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Validation of the Remote Food Photography Method to Quantify Intake of Infant Formula

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VALIDATION OF THE REMOTE FOOD PHOTOGRAPHY METHOD
TO QUANTIFY INTAKE OF INFANT FORMULA

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Human Ecology

by
Abby Francis Duhé
B.S., Louisiana State University, 2011
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LIST OF ACRONYMS

%	=	Percent
$^2\text{H}_2\text{O}$	=	Water isotope
$^3\text{H}_2\text{O}$	=	Water isotope
AAP	=	American Academy of Pediatrics
BMI	=	Body mass index
cm	=	Centimeter
CO_2	=	Carbon dioxide
DLW	=	Doubly labeled water
DWF	=	Directly Weighed Foods
EMA	=	Ecological momentary assessment
FDA	=	Food and Drug Administration
FFDCA	=	Federal Food, Drug, and Cosmetic Act
FFQ	=	Food frequency questionnaire
fl oz	=	Fluid ounce
FTO	=	Fat mass and obesity associated gene
g	=	Gram
H_2^{18}O	=	Water isotope
HIPAA	=	Health Insurance Portability and Accountability Act
ICC	=	Intra-class correlation coefficient
IRB	=	Institutional Review Board
kcal	=	Kilocalorie
kg	=	Kilogram
kJ	=	Kilojoule
lb	=	Pound

m ²	=	Meter squared
MANOVA	=	Multivariate Analysis of Variance
MC4R	=	Melanocortin 4 receptor
mL	=	Milliliter
NHANES	=	National Health and Nutrition Examination Survey
NIH	=	National Institutes of Health
PBRC	=	Pennington Biomedical Research Center
PLU	=	Price look up
RFPM	=	Remote Food Photography Method
SD	=	Standard deviation
USDA	=	United States Department of Agriculture
WHO	=	World Health Organization

ABSTRACT

Childhood obesity rates have more than tripled since the 1970s, and this increased prevalence is cause for concern as childhood obesity increases the risk of adult obesity and other comorbid diseases^{1,2}. Evidence suggests that the origins of obesity can be identified in infancy³. Accurate methods of assessing food intake in infants can be utilized to establish effective feeding practices in infancy and to assess the relationship between infant feeding practices and the risk of childhood obesity⁴⁻⁶. Current methods are either subjective⁷ or have limited ability for widespread use beyond clinical research settings due to cost and high burden^{8,9}. The aim of the Baby Bottle study was to assess the accuracy of the Remote Food Photography Method (RFPM), a novel food intake assessment method, in estimating infant formula as compared to the gold standard, the directly weighed foods method. In the Baby Bottle study, fifty-three adults were recruited to prepare infant formula bottles and use the RFPM to capture photographs of infant formula at different stages of bottle preparation. Dry food provision, liquid food provision, and liquid waste gram weights measured by the RFPM and directly weighed foods method were compared to assess the accuracy of the RFPM in the estimation of infant formula. Paired dependent t-tests and the Bland-Altman regression method were employed to determine if the weight estimations of RFPM differed from the weights measured by the directly weighed foods method. Multivariate analysis of variance was used to analyze the effects of trial number and caregiver status on infant formula preparation. The RFPM estimated liquid formula intake within 10% of the directly weighed foods method, with error of $-4.1 \pm 14.4\%$ ($P < 0.0001$), $2.8 \pm 16.3\%$ ($P = 0.1550$), and $7.0 \pm 12.4\%$ ($P < 0.0001$) in 2 fluid ounce, 4 fluid ounce, and 6 fluid ounce bottles, respectively. The RFPM overestimated liquid formula intake by $14.0 \pm 10.3\%$ ($P < 0.0001$) in 8 fluid ounce bottles. There were no significant differences between individuals in the caregiver group ($n = 28$) and the non-caregiver group ($n = 25$) based on all demographic and descriptive characteristics. There were no significant differences for the effects of trial number and caregiver status on infant formula preparation except for a significant main effect of caregiver status on the preparation of dry food provision of 2 fluid ounce bottles ($P = 0.0499$) and a significant interaction between trial number and caregiver status on preparation of dry food provision of 4 fluid ounce bottles ($P = 0.0146$). In conclusion, the RFPM is a viable method of measuring infant formula intake as it provides more valid estimates as compared to commonly used self-report methods in clinical practice and research and decreased cost, burden, and time commitment from individuals as compared to current objective methods^{8,9}.

CHAPTER 1: INTRODUCTION

Introduction

According to the most recent National Health and Nutrition Examination Survey (NHANES) from 2009 to 2010, 16.9% of children and adolescents aged 2 to 19 years were obese and 31.8% were overweight and obese². Additionally, these data showed that almost 10% of infants and toddlers aged 6 to 23 months were obese defined as weight for recumbent length greater than or equal to the 95th percentile². Evidence suggests that the origins of obesity can be identified in the first years of life³. Parents and other family members are responsible for influencing infant feeding behavior^{4,8,10} and establishing the foundation for a healthy diet and lifestyle⁴. A culmination of studies that assessed the energy requirements in children using the doubly labeled water method suggests that childhood obesity can be best explained by an increased energy intake¹¹. Certainly, breastfeeding, timing of solid food introduction, and home food environment during early childhood can impact the risk of childhood obesity^{10,12}. Assessing food intake in infants is useful in monitoring growth and development, but it also has the potential to be instrumental in preventing overfeeding in infancy¹³ and, therefore, minimizing the risk of childhood obesity^{10,12}.

Measuring food intake for infants is challenging because foods and eating patterns are constantly changing during the first two years of life. In addition, food intake during the first two years of life dramatically differs from food intake during the remainder of life¹⁴, where most of the available methods for assessing food intake are focused. Infant nutrition begins with exclusive feeding of either human milk or infant formula, or a combination of human milk and infant formula from birth until six months of age. Pureed foods and, then, solid foods are introduced usually beyond six months so infants are consuming a mixed diet near the end of the first year of life^{4,15}.

Current methods for measuring infant food intake include the directly weighed foods method, test weighing, the doubly labeled water method, estimated food diaries, twenty-four hour diet recalls, and food frequency questionnaires. The directly weighed foods method is considered one of the most common reference standards as it is one of the most accurate and direct methods for measuring food intake in infants^{9,16}. Test weighing and the doubly labeled water method have been shown to overestimate food intake within 10% in infants when compared to the directly weighed foods method¹⁷⁻²⁰. The twenty-four hour diet recall method has been shown to overestimate food intake by 13% in infants as compared with the directly weighed foods method¹⁴. Andersen and colleagues developed a food frequency questionnaire that overestimated

food intake by 25% as compared to the directly weighed foods method¹⁶. Establishing accurate methods to estimate food intake in infants is important for establishing effective feeding practices, supporting adequate growth and development and understanding the role of food intake during infancy in the development of childhood obesity.

The Remote Food Photography Method (RFPM) is an emerging method for assessing food intake that utilizes digital photography of food provision and plate waste to estimate food intake^{7,21}. With the RFPM, individuals take photographs of food provision and plate waste using the SmartIntake© application developed at Pennington Biomedical Research Center^{21,22}. Then, photographs are transmitted in near real-time over the wireless network and are analyzed using digital photography where food photographs are compared to standard food portions and linked to the foods' nutrient information in order to obtain food gram weights, macronutrient content, and micronutrient content^{7,9,21}. There are several advantages of the RFPM as compared to other methods including reduced patient burden and elimination of the need for individuals to estimate portion size²². Another strength of the RFPM is that the use of reminder message prompts helps to minimize missing data and to promote data quality²¹. The RFPM has been validated in free-living adult individuals²¹, and it has the potential to be a useful tool for assessing food intake in other populations including infants in research and clinical settings²¹.

Objectives

The primary objective of the Baby Bottle study was to:

1. assess if the RFPM can accurately estimate simulated infant formula intake compared to the gold standard– the directly weighed foods method.

Secondary objectives were to:

2. evaluate the inter- and intra-individual variability in infant formula preparation and
3. investigate the variability in infant formula preparation between caregivers and non-caregivers of infants. A caregiver was defined as an individual who identified as a parent, grandparent, sibling, aunt or uncle, or nanny or babysitter who has provided care to an infant within the last twelve months.

Justification

The American Academy of Pediatrics (AAP) has provided recommended ranges for feeding infants to help caregivers and health providers ensure energy intake is sufficient to

support infant growth without overfeeding (Table 1)²³. As illustrated in Table 1, the amount of each feeding and frequency of feedings per day increase with age to provide a steady increase in energy intake (kcal/day), which is necessary to promote growth. Given the recommended feeding patterns (and expected energy intake) throughout the first six months of life, a goal of the Baby Bottle study was to evaluate the capacity of the RFPM to estimate energy intake for bottles of infant formula prepared with a final volume of 2 fl oz, 4 fl oz, 6 fl oz, and 8 fl oz.

Table 1: American Academy of Pediatrics Recommendation for Infant Feeding					
Age of Infant	Feeding Size (oz)	Energy intake (kcal)	Total Feedings (per day)	Total Amount/Day	Total energy intake (kcal/day)
Newborn	2 fl oz	40	6	12 fl oz	240
1 month	3 fl oz	60	6	18 fl oz	360
2 months	4 fl oz	80	6	24 fl oz	480
4 months	6 fl oz	120	4	24 fl oz	480
6 months	8 fl oz	160	4	32 fl oz	640

In order to understand the clinical significance of RFPM measurement error in estimating food intake from bottles of infant formula, the total daily energy intake that would be either over- or under- estimated if the measurement error was 5%, 10%, 15% or 20% for standard meal sizes commensurate with recommendations from birth to six months was calculated (Table 2). An estimated food intake using the RFPM that has 5% measurement error for a newborn, where the feeding size is 40 kcal, would result in a difference of 3 g per meal in formula or a difference of ± 12 kcal per day. Similarly for an infant aged six months, a measurement error of 5% would yield a difference of ± 32 kcal per day.

Based on current objective methods for measurement of infant food intake, the goal of the RFPM method in estimating infant formula intake is within 10% of actual measured energy intake. This error is supported by previous work that validated the RFPM for assessment of food intake in adults and validation studies in comparison to the directly weighed foods method of other commonly used methods for evaluating infant food intake. If the RFPM method is shown to provide estimates of energy intake within 10% of actual measured energy intake, it will be demonstrated that the method can be applied to the estimation of infant formula intake and,

importantly, that RFPM may provide a more valid approach for quantifying infant food intake compared to commonly used self-report methods in clinical practice and research.

Table 2: Measurement Error on Infant Formula Preparations					
Age of Infant		5%	10%	15%	20%
Newborn	g difference/ feeding	3.05	6.1	9.15	12.2
	kcal difference/ feeding	2	4	6	8
	kcal difference/ day	12	24	36	48
1 month	g difference/ feeding	4.575	9.15	13.725	18.3
	kcal difference/ feeding	3	6	9	12
	kcal difference/ day	18	36	54	72
2 months	g difference/ feeding	6.1	12.2	18.3	24.4
	kcal difference/ feeding	4	8	12	16
	kcal difference/ day	24	48	72	96
4 months	g difference/ feeding	9.15	18.3	27.45	36.6
	kcal difference/ feeding	6	12	18	24
	kcal difference/ day	24	48	72	96
6 months	g difference/ feeding	12.2	24.4	36.6	48.8
	kcal difference/ feeding	8	16	24	32
	kcal difference/ day	32	64	96	128

Limitations

Limitations of this study were:

1. Whole milk powder as compared to powdered infant formula was used in bottle preparation in the Baby Bottle study, as it was a cost effective substitute for commercial powdered infant formula. To prepare 159, 2 fluid ounce, 159, 4 fluid ounce, 159, 6 fluid ounce, and 159, 8 fluid ounce bottles, 22 Similac Advance containers costing approximately \$550 would need to be purchased. In comparison, an equivalent amount of whole milk powder costs approximately \$125. Since the Baby Bottle study design involved discarding the prepared bottles without providing the prepared bottles to infants for feeding, it was wasteful to spend \$550 for Similac Advance containers. The key assumption for the use of whole milk powder as a substitute for infant formula was that the consistency of the whole milk powder and powdered infant formula are the same. Importantly, the participants were unaware of the powder substitution as a 1.45 lb container of Similac Advance formula was purchased and continually refilled. Using the commercial container, participants were able to use the same standard infant formula

scoop provided with the Similac Advance infant formula container and preparation instructions on the back of the Similac Advance infant formula container.

2. The pattern in which individuals prepare bottles was standardized, as the study design required weighing the bottles at each step. Study participants were required to prepare bottles by adding dry powder followed by water as compared to water followed by dry powder. It was necessary to weigh the dry powder to assess the accuracy of the RFPM in estimating the energy content of the dry powder. In free-living conditions, it is unknown the manner in which an individual prepares a bottle. To minimize the effect of this limitation, participants were encouraged to read and interpret the instructions for infant formula preparation provided on the Similac Advance infant formula container.

Assumptions

Assumptions in this study were:

1. The sample size was viable to reflect the relationship between the RFPM and the directly weighed foods and estimated food intake. Other statistical assumptions include a power of 0.80 for sample size estimation and an alpha equal to 0.05 for statistical analysis.
2. The randomization of discarding prepared infant formula was determined using a random number generator reflecting a Gaussian distribution (Mean=0.80, SD=0.20). The range of discarding prepared infant formula is assumed to reflect the infant formula waste of typical infant feeding. All numbers over 100% that were generated were assumed to be 100%.

CHAPTER 2: REVIEW OF LITERATURE

Childhood Obesity

There is no argument that the increased prevalence of overweight and obese adults worldwide is cause for concern. More alarming, however, is the rapid increase in overweight and obesity in children. Data from the National Health and Nutrition Examination Survey (NHANES) from 1971 to 1974 showed that 5% of children and adolescents aged 2 to 19 years were obese¹. According to the most recent NHANES data from 2009 to 2010, 16.9% of children and adolescents aged 2 to 19 years were obese and 31.8% of children and adolescents aged 2 to 19 years were overweight and obese². These data suggest that since the 1970s, childhood obesity rates have more than tripled^{1,2}. Rates of obesity in infants and toddlers have also increased during this timeframe^{1,2}. The 2009-2010 NHANES data showed that 9.7% of infants and toddlers aged 6 to 23 months were obese defined as weight for recumbent length greater than or equal to the 95th percentile².

Evidence suggests that the origins of obesity can be identified in early childhood³. Families and immediate caregivers hold the largest influence on the health behaviors of young children⁶. Breastfeeding, timing of solid food introduction, and home food environment during early childhood can impact the risk of childhood obesity^{10,12}. As a consequence of the rise of childhood obesity, other chronic comorbidities including hypertension, type 2 diabetes, asthma, and dyslipidemia are also on the rise and are impacting the long-term health of children^{24,25}. The prevalence of childhood hypertension has increased since the late 1980s²⁴, and obese children are 2.5-3.7 times more likely to have hypertension than non-obese children^{12,26}. In adults, type 2 diabetes has consistently been correlated with obesity²⁷, and weight status has also been shown to affect the incidence of type 2 diabetes throughout childhood⁶. Furthermore, overweight and obesity throughout childhood and adulthood have been associated with a twelve-fold increase in the development of type 2 diabetes²⁸. In addition to hypertension and type 2 diabetes, asthma is influenced by obesity and weight status in children^{3,29,30}. According to a longitudinal study on childhood obesity and asthma, higher weight status was associated with asthma severity and poor asthma control²⁹.

There is strong evidence suggesting that overweight and obesity in children increases the risk for obesity and comorbidity in adulthood³¹⁻³⁴. Furthermore, the existence of cardiovascular risk factors in childhood, such as obesity, dyslipidemia, hypertension, and type 2 diabetes, contributes to the development of cardiovascular disease in adulthood^{12,34,35}. For example, it has been shown that dyslipidemia throughout childhood continues into adulthood in 50% of cases¹².

Since cardiovascular disease is the leading cause of death in the United States, strategies to reduce overweight and obesity in children can be effective steps to reduce disease risk and healthcare burden³⁵.

There is increasing evidence that genetic factors also affect the risk of obesity. Research suggests that parental weight status influences the weight of offspring with maternal weight having the strongest association³⁶. Some single-gene defect disorders including Prader-Willi syndrome and Bardet-Biedl syndrome have presented central obesity as a primary clinical feature which affects about 5% of childhood obesity cases³⁷. Furthermore, there is evidence of a genetic predisposition for obesity with certain genes including fat mass and obesity associated gene (FTO) and melanocortin 4 receptor (MC4R) as two of the most studied genes with associations to body mass index, adiposity, and obesity^{37,38}. Obesity may be explained in part by genetic factors, but, ultimately, it is the result of a chronic imbalance between energy intake and energy expenditure³⁵. A positive energy balance, whether achieved through increased energy intake or reduced energy expenditure, contributes to weight gain and has the potential to lead to overweight and obesity³².

Determinants of Obesity in Children

Energy Balance

The existence of obesity is directly influenced by a positive imbalance of energy intake and energy expenditure. Positive energy imbalance may be the result of high energy intake, low energy expenditure, or a combination of both³², and this relationship is responsible for weight gain³⁹. According to the first law of thermodynamics, energy cannot be destroyed; it can only be transferred or stored^{40,41}. The concept of energy balance follows the first law of thermodynamics because it involves energy intake and energy expenditure and their direct relationship to each other and to the amount of energy stored in the body. The energy balance equation is defined as *energy intake = energy expenditure*, but energy balance is more commonly referred to as *energy intake + energy expenditure = energy stores*³⁹.

Energy intake refers to the energy derived from the intake of the 3 primary macronutrients—carbohydrate, protein, and fat⁴². Energy expenditure reflects total energy expended during a day which includes resting energy expenditure, the thermic effect of food or diet-induced thermogenesis, and energy expended from physical activity and all non-exercise activities⁴⁰.

The amount of energy needed to sustain normal bodily functions and to maintain body mass is termed the energy requirement⁴². The energy requirement of a free-living individual can be measured accurately during weight stability by the doubly labeled water method (DLW). While DLW data is available to scientists and may be used by some clinical professionals, data on energy requirements using the DLW method is not widely available to the general public. Consequently, many adults cannot accurately estimate the energy requirement for themselves or for their families.

Energy balance studies in children are complex, given the additional variability of growth and the evidence that rapid growth during childhood can lead to obesity during adulthood³⁹. For infants, energy requirements include the energy cost of growth, physical activity and movement. Higher rates of weight gain in infancy is associated with an increased risk of obesity¹ and is one of the strongest risk factors for childhood obesity¹³. This has been widely reported in industrialized countries where formula feeding often outweighs breastfeeding, and it may be due to feeding mode since formula fed infants, typically, gain weight faster than breastfed infants¹. Additionally, more rapid weight gain in formula fed infants may be attributed to the fact that formula feeding mothers tend to follow feeding schedules rather feeding on demand which may result in overfeeding¹³. The link between rapid growth in infancy and obesity in adulthood deserves further investigation.

Energy expenditure, including physical activity, is an important aspect in maintaining energy balance and preventing excess weight gain in children³. Physical activity is also essential for normal growth and development in children³². Recently, physical inactivity has been evidenced in children, and current Western civilization standards have perpetuated this physical inactivity³². Physical inactivity may contribute to excess weight gain and obesity in childhood, and, in consequence, the risk of obesity in adulthood³². Sedentary activities including television viewing, persistent computer use, and other electronic media use are linked to the risk of childhood obesity^{32,43,44}. The American Academy of Pediatrics (AAP) recommends that children younger than two years refrain from television viewing and that children two years of age or older limit television viewing to no more than two hours per day⁴⁵. Energy expenditure in infants consists of the cost of growth, physical activity, and movement, and it should increase throughout infancy to promote normal growth and development^{46,47}. Parents and caregivers are encouraged to expose infants to active play to stimulate movement and limit time when the infant's movement is restricted as in car seats or strollers^{48,49}. Caregivers of children at all ages should provide a safe and structured play environment including outdoor exploration and other sources of activity^{32,45,49}.

Children should be a priority in advocating for the prevention of obesity and other diseases providing a focus that is on prevention rather than on treatment of childhood obesity⁴³.

Research suggests that increased food intake rather than decreased physical activity is responsible for the increased rates of overweight and obesity in both adults and children¹¹. While increasing the quantity of food in childhood clearly has a role in affecting weight gain and obesity rates, the quality of the diet in childhood is also important to support growth, development, and can establish the eating behaviors adopted in adulthood⁵⁰. For example, fruit and vegetable exposure and consumption during childhood has been shown to improve fruit and vegetable consumption in adulthood^{15,51}. A small percentage of children are meeting fruit and vegetable recommendations⁵⁰, identifying a common problem in children's diet composition. Highly processed foods containing sodium such as marketed snack foods are often provided to young children including infants and toddlers as these snack foods are appetizing and easy to consume²⁴. Individuals as young as six years are reported to have sodium intakes above the United States Department of Agriculture (USDA) recommendations²⁴. High intake of sodium, including during early childhood is associated with risk of hypertension⁵². Maintaining sodium intake within USDA recommendations may be beneficial in preventing or controlling hypertension²⁴. During the last few decades, there has been an increase in consumption of processed foods and sugar-sweetened beverages due to their affordability, durability, and convenience. Providing highly processed energy dense snacks to young children including infants affects dietary composition and, likely, preferences throughout childhood and into adulthood^{15,50}. Frequent consumption of highly processed foods has been linked to weight gain and increased risk of chronic disease^{53,54}. Typically, processed foods contain low amounts of vitamins, minerals, and fiber and high amounts of added sugars and sodium^{54,55}. In the late 1970s, high fructose corn syrup became a popular and economical sweetener leading to the rise of refined sugar consumption⁵⁶. Consumption of sugar-sweetened beverages including soft drinks has increased especially in children and adolescents leading to increased concern about childhood obesity^{24,57}. Increased intake of sugar-sweetened beverages increases weight gain and the risk of dental caries. Consequently, increased weight gain in childhood resulting from poor diet quality and increased food intake increases the risk of obesity, cardiovascular disease, type 2 diabetes, and metabolic syndrome in later life⁵⁸⁻⁶⁰.

Nutritional Programming of Infants

The most "critical" period of nutritional programming begins while the fetus is growing in utero and continues through the first two years of life^{4,61}, recently referred to as the first 1000

days⁶². Before birth, maternal diet is responsible for providing energy and nutrients to the growing fetus⁴. The fetus is exposed to the nutrients of the maternal diet and other metabolic and environmental factors and contaminants through the amniotic fluid⁴, and the composition can positively or negatively affect the fetus. Previous literature has shown that gestational weight gain, gestational diabetes mellitus, and tobacco use during pregnancy are significant factors that may negatively affect infant birth outcomes and early growth and development^{13,36}. When a mother chooses to breastfeed after birth, the infant continues to be exposed to the maternal diet further linking the fetal and the growth environments of early life⁴. The nutritional environment that parents and caregivers provide exposes infants to immediate effects and nutritional programming for long-term effects⁴. Important nutrition decisions during this “critical” period include the decisions about the initiation of breastfeeding, the duration of breastfeeding, and the use of formula feeding. It has been shown that infants who are exclusively bottle-fed may lack the self-regulation skills^{4,5} to prevent overfeeding⁶. Infant-initiated bottle emptying during the first six months of life has been associated with excess weight gain in the first year of life⁶³. Another critical decision for parents and caregivers during the first year of life is the timing of the introduction of solid foods^{64,65}. Parents and caregivers are responsible for nutritional programming for growth and development and setting the foundation for a healthy life.

Infant Food Intake

The characteristic rapid growth and development of infants causes eating patterns to constantly change throughout the first two years of life⁴. During this critical growth period, infants are constantly developing and learning new feeding skills⁴. Food intake during the first two years of life dramatically differs from food intake during the remainder of life¹⁴. Infant nutrition begins with exclusive feeding of either human milk or infant formula, or a combination of human milk and infant formula. Pureed foods and, then, solid foods are introduced gradually within the first year of life^{4,15}. Early introduction of cow’s milk, high juice intake, and low intake of fruits and vegetables during the early period of solid foods introduction have been shown to be associated with overweight and obesity in childhood⁶⁵. Early feeding practices shape long term eating behaviors so the quantity and variation of foods during infancy is important¹⁰. Parents and caregivers are responsible for influencing feeding behavior^{4,8,10} and establishing the foundation for a healthy diet and lifestyle⁴.

Human milk is the ideal nutrition for infants as it is specifically designed for human infants⁵. Breastfeeding promotes attachment between mother and infant, and it has nutritional and

immunological advantages for the infant and mother⁵. Exclusive breastfeeding and long duration rates are argued by some groups to be protective against childhood obesity^{5,66,67}. With respect to feeding behavior, breastfeeding promotes infant self-regulation of feeding^{4,5} which may reduce the likelihood of overeating and weight gain⁶. The World Health Organization (WHO) recommends exclusive breastfeeding for six months, but supports that partial breastfeeding and shorter durations of breastfeeding can still have beneficial effects on growth and health in infants⁶⁸. In a recent study on feeding patterns in the first two years of life, exclusive breastfeeding was significantly associated with higher weight, higher length, lower probability of stunting, lower probability of wasting and lower probability of infections⁶⁸.

For infants who are not exclusively breastfed by choice or necessity, commercial infant formulas are the best alternative⁶⁸. According to the Federal Food, Drug, and Cosmetic Act (FFDCA), infant formula should be used solely as food for infants as a complete or partial substitute for human milk⁶⁹. The majority of infant formula available in the United States is sold in powdered form. Caregivers mix powdered formula with water to prepare formula for infants to consume. While the United States Food and Drug Administration (FDA) regulates the nutrient content of infant formula, there may be variability in formula intake with differences in formula preparation by caregivers and infant feeding patterns⁶⁹.

Measuring food intake in infants can be challenging due to the constant changes in eating patterns and large variability in food selection. This is especially true with infants who cannot communicate hunger and satiety needs as easily as older children and adults. Challenges and inconsistencies with measuring food intake in infants include losses from spit up and movement during feeding⁷⁰. Regarding breastfeeding, typical measurement cannot be accomplished as babies usually feed directly from the breast and the baby's self-regulation determines the duration of feeding in most cases. Establishing accurate methods to assess food intake in infants is important for establishing effective feeding practices, supporting adequate growth and development and understanding the role of infant food intake in the development of childhood obesity.

Measurement of Infant Food Intake

There are several available methods for measurement of food intake in infants (Table 3). Current methods for quantifying infant food intake have advantages and disadvantages, and differing methods can be useful in varying situations. The objective methods of measuring food intake in infants include the directly weighed foods method, test weighing, and the doubly labeled

water method. In brief, the objective methods are highly accurate but have reasonably high burden and cost^{8,9}. In infants, significant error may occur with these methods from losses due to spit up and typical infant movement⁷¹. Subjective methods commonly referred to as self-report methods include estimated food diaries, twenty-four hour diet recalls, and food frequency questionnaires. Self-report methods for quantifying food intake are relatively simple to execute, but, with the care of infants, rely on an caregiver's memory for identifying food intake and portion estimation⁷.

Table 3: Summary of Methods Used in Quantifying Infant Food Intake				
	Accuracy	Burden	Cost	Dissemination
Directly Weighed Foods ⁹	High	High	Medium	Low
Test Weighing ^{18,72}	High	High	Medium	Low
Doubly Labeled Water ⁷³	High	Medium	High	Low
Estimated Food Diary ²⁰	Medium	High	Medium	Medium
24 Hour Diet Recall ¹⁴	Low	Low	Medium	Medium
Food Frequency Questionnaire ^{16,74,75}	Low	Low	Low	High

Objective Methods

Directly Weighed Foods

Regarded as one of the most accurate methods for measuring food intake^{9,16}, the directly weighed foods method is a reference method of measuring food intake that does not depend on memory and is easy to apply to infants of varying ages¹⁶. As the name suggests, the directly weighed foods method involves weighing all food items before and after consumption. Weights of food provision and plate waste are recorded so that food intake can be calculated by subtracting the weight of plate waste from food provision¹⁹. Ideally, scales that are accurate to one gram are utilized in the directly weighed foods method^{20,74}. Descriptions of food items or foods not consumed may be necessary to maintain accurate estimation¹⁶. Other strengths include that the method is non-invasive and relatively inexpensive as compared to the doubly labeled water method. Conversely, the directly weighed foods method is also considered time consuming and burdensome to weigh individual food items and plate waste⁹. While the directly weighed foods method accurately assesses current consumption, it may underestimate habitual consumption as individuals being asked to weigh food for assessment of food intake may influence usual food intake behavior and alter what and how much food is being consumed¹⁴.

When assessing infant formula intake, the procedure involves weighing the dry formula, the liquid formula (after mixing with water), and the formula waste²⁰. Infant formula bottles can be weighed directly using this method so actual intake can be assessed, but there may be minor overestimation due to losses from spit up, spillage during feeding, or drool⁷².

In a comparison of methods to assess infant food intake, Fisher and colleagues determined that the directly weighed foods method estimated energy intake as 740 ± 154 kcals, which was within 5% of estimated energy requirements^{14,42}. The directly weighed foods method is, therefore, often used as the validation standard for assessing food intake in infants. Examples include Borschel and colleagues¹⁷ who compared test weighing and Butte et al⁷⁶ who compared the doubly labeled water method to the directly weighed foods method in infants^{17,76}, respectively.

Test Weighing

Test weighing is an effective method developed to quantify milk intake in both breastfed and formula fed infants, and it can be used in both clinical practice and research^{18,70}. Test weighing has been shown to be the best method for assessing energy intake in breastfed infants^{17,71,72}. Although less common, test weighing can also be utilized in measuring intake in formula fed infants, but measurement of infant formula intake can be more directly obtained through the directly weighed foods method¹⁷.

The procedure for test weighing involves weighing the infant before and after an observed feeding with the difference in body weight approximating food intake^{17,71,72}. Test weighing can be an advantageous method of quantifying energy intake because it is simple to perform and can be utilized in clinical research, clinical practice, and home settings^{18,72}. A principal strength of the test weighing method is that it can be applied to infants who are exclusively breastfed, as it does not disturb normal feeding practices⁷². One weakness of test weighing is that there may be difficulty in detecting small differences in body weight, especially in young infants when the volume of milk consumed is also small^{18,71,77}. Previous studies have emphasized the importance of using a scale with sufficient accuracy to detect small weight changes as small as one gram^{71,78}. In addition, insensible water losses due to inconsistency with clothing changes, evaporation from the skin⁷⁹, losses from spit up, and infant movement can be weaknesses to test weighing. It has been estimated that insensible water losses during infant feeding approximate 3% of food intake^{17,80}. Haase and colleagues have shown that the best way to account for insensible water losses and infant movement is by tightly swaddling infants and

standardizing clothing⁷¹. Previous studies state that test weighing can be an accurate method for clinical research if measurements are consistent, electronic scales are used, movement is limited, and losses are accounted for^{18,71}. These studies emphasize the importance of consistency with scales, tightly swaddling infants before weighing, and including diapers and blankets for both before feeding and after feeding weights^{18,71}.

Several studies have investigated the accuracy of the test weighing method against the directly weighed foods method. First, Borschel and colleagues compared the accuracy of the two methods in infants from birth to six months of age who were being formula fed¹⁷. The volume of infant formula intake from test weighing (Range 737-847 mL/day) did not differ significantly from the infant formula intake volume measured from the directly weighed foods method (Range 861-929 mL/day). Test weighing underestimated the directly weighed foods method by 10% in infants aged one month, 13% in infants aged two months, 9% in infants aged four months, and 7% in infants aged six months¹⁷. The overall mean difference in formula intake between the two methods was 16 ± 2 mL per feeding or an average underestimation of the test weighing method as compared to directly weighed foods¹⁷. In addition, Meier et al studied test weighing against the directly weighed foods method. Test weighing on infants was completed using mechanical and electronic scales, and the formula provided to the infants was directly measured as the reference standard. As compared with the directly weighed foods method (33.1 mL/feeding), test weighing using a mechanical scale (35.6 mL/feeding) overestimated food intake by 8%, and test weighing using an electronic scale (33.4 mL/feeding) overestimated food intake by only 1%^{18,72}. Savenije and Brand argue that infant scales may not be sensitive enough to determine the small changes in infant weights after feeding⁷⁷. Although there has been conflicting reports on the level of accuracy of the test weighing method against validation standards, the majority of evidence supports the use of test weighing as an accurate assessment method when procedures are standardized, infants are tightly swaddled, and sensitive scales are used for detection^{18,71}.

Doubly Labeled Water

The doubly labeled water (DLW) method is considered the gold standard for measuring energy requirements in free-living weight-stable individuals⁷, and it has been applied to both formula fed and breastfed infants⁴⁶. For infants, the DLW method can be used to measure total energy expenditure, milk intake, total energy intake, and energy content of milk. DLW is a non-invasive and safe method of estimating total energy expenditure in free-living individuals including infants⁷³. The use of DLW in infants for measurement of energy expenditure has been

validated against indirect calorimetry in infants⁸¹. Indirect calorimetry for infants requires the infants to be placed into a hospital head box in a room drawing air at a known constant rate. This can be burdensome for infants who may be fussy from being alone or away from their parents for long periods of time⁸¹. In addition, indirect calorimetry cannot be considered a free-living measurement.

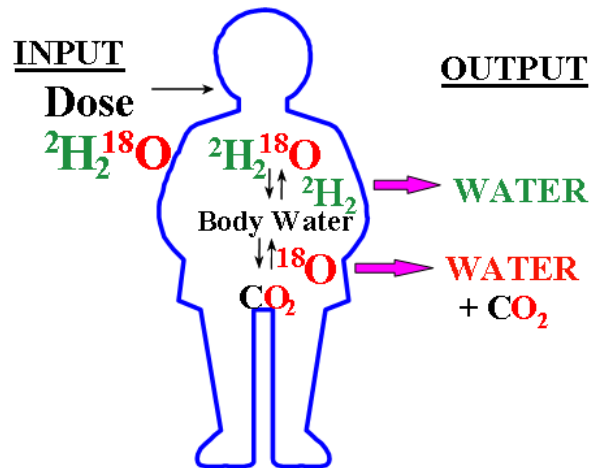


Figure 1: Schematic of the Doubly Labeled Water Method

The general procedure of the DLW method (Figure 1) in infants is that two isotopes of water (H_2^{18}O and $^2\text{H}_2\text{O}$ or $^3\text{H}_2\text{O}$) are administered to the infant and the disappearance rates of the isotopes are monitored in the saliva or urine. As shown in Figure 1, the disappearance rate of $^2\text{H}_2\text{O}$ or $^3\text{H}_2\text{O}$ provides water output and the disappearance rate of H_2^{18}O provides water output and carbon dioxide (CO_2) production. The difference of the two disappearance rates provides CO_2 production. The measure of CO_2 production, in addition to the respiratory quotient for the specific individual, provides total energy expenditure^{73,81}. Typically, an infant is weighed prior to the DLW procedure and the isotope doses given are relative to body weight. Doses are prepared and administered to the infant using bottles, syringes, or feeding tubes⁸². After dose administration, urine or saliva samples are collected periodically from the infant to determine the disappearance rates of the two isotopes. Previous studies vary in the length of sample collection from five to fourteen days⁸¹. In energy balance, the DLW method provides energy expenditure, which is equal to energy intake⁷.

Roberts et al compared the DLW method to indirect calorimetry for preterm infants between six and seven months of age. DLW and indirect calorimetry were performed for five days on the infant participants. The DLW significantly overestimated water intake by $5.7 \pm 1.4\%$

($P < 0.05$) in comparison to indirect calorimetry, but values for CO_2 production, energy expenditure and metabolizable energy were not significantly different from the values using indirect calorimetry⁸¹. Butte and colleagues compared the DLW method to other validation standards—the directly weighed foods method in formula fed infants and the test weighing method in breastfed infants. For the DLW procedure, infants were dosed on day one of the experiment using a pre-weighed syringe, and urine samples were collected daily for fourteen days. Results showed that DLW overestimated intake by an average of 14% in breastfed infants as compared to intake measured using the test weighing method and 8% in formula fed infants as compared to the intake measured using the directly weighed foods method. After adjusting estimates from breastfed infants for environmental water influx and insensible water loss, the relative bias decreased to 5%, and after adjustment for environmental water influx for formula fed infants, the relative bias decreased to 1-2%⁷⁶. Davies et al compared the DLW method to directly weighed foods in preschool age children from one to five years. Urine samples were collected for ten days after DLW dosing, and parents or caregivers of children completed the directly weighed foods method for assessing food intake for five days within the DLW sample collection period. In the subgroup of children under two and one-half years of age, DLW underestimated energy intake by 6%¹⁹. Lanigan further studied the comparison of the DLW method and the directly weighed foods method in infants aged six to twelve months. Doubly labeled water with seven day urine sample collection underestimated mean energy intake by 7.3% as compared to directly weighed foods records²⁰. Obvious limitations of the DLW technique include cost of the isotopes and analysis, moderate subject burden, difficulty of obtaining urine and saliva samples, and technical availability of mass spectrometry instrumentation. These disadvantages lessen the likelihood for widespread scalability of this method beyond clinical research settings^{7,8}.

Subjective Methods

Estimated Food Diary

The estimated food diary method is a popular self-report method of food and nutrient assessment⁷. The food diary procedure for estimating food intake requires the individual to record details of each food and drink consumed for a specified time period to predict typical intake. This is usually done using pen and paper, and researchers and clinicians may opt to provide a food diary template to improve data quality²⁰. Individuals are instructed to record the date, time, all foods and drinks consumed, the amounts of food and beverages provided, and the amounts of

food and beverages not consumed²⁰. In addition, portion sizes, recipes, individual ingredients, and preparation instructions should be included for completeness²⁰.

The estimated food diary has been compared to the directly weighed foods method, and it is commonly used as a simple and inexpensive alternative to the directly weighed foods method²⁰. Portion size estimation is crucial in this assessment method, and it represents a major weakness of estimated food diaries, as most individuals cannot accurately estimate portion sizes⁷. Another weakness is that there is potential for the use of estimated food diaries to cause under eating during the test time period²¹. Missing data may also occur when using this method as estimated food diaries are usually kept for several days²¹. Strengths of this method include that there is no reliance on patient memory⁸³ and that estimated food diaries may be representative of habitual food intake²⁰.

Lanigan et al studied the possibility of the estimated food diary records as an alternative for directly weighed foods records. In this study, dietitians trained parents and caregivers on using the food diary method including portion size estimation using standard household measures as tools²⁰. Estimated food diaries underestimated food intake by 3.6% (mean bias of 138 kJ/day) compared to the directly weighed foods method²⁰. In this study, there was no significant difference between mean energy intake from estimated food diaries and the directly weighed foods method²⁰. Lanigan and colleagues support the use of estimated food diaries as an accurate alternative to the directly weighed foods method²⁰.

Twenty-Four Hour Diet Recall

The twenty-four hour diet recall represents another self-report method⁷ that is typically used on a large scale, and its accuracy is not well documented¹⁴. Twenty-four hour diet recalls are used to report food and beverage consumption in the previous twenty-four hours⁸³. Typically, a trained individual interviews the patient in person or through a telephone interview¹⁴. It relies on the participant to accurately estimate portion sizes and recall foods and drinks consumed and amounts consumed⁷.

Strengths include ease of use, low participant burden, and low cost¹⁴. Weaknesses are that the twenty-four hour diet recall method relies on participant memory and portion estimation⁸³, and the method is usually not representative of habitual dietary patterns since only the previous twenty-four hours are reported¹⁴. Portion size estimation has been shown to be a significant source of error in twenty-four hour diet recalls¹⁴. In addition, individuals may forget to include

sauces, condiments, drinks, and snacks between meals, which may cause misreporting of food intake⁸³.

Fisher et al examined the use of a telephone administered multiple pass twenty-four hour recall against a three day directly weighed foods record¹⁴. In this study, trained study dietitians performed twenty-four hour diet recalls by telephone to assess infant food intake. The twenty-four hour diet recall method overestimated energy intake by 13% among the infants aged seven to eleven months as compared to the directly weighed foods method¹⁴.

Food Frequency Questionnaire

The food frequency questionnaire is a commonly used self-report method of assessing energy and nutrient intake^{74,75}. It has been shown to be the most appropriate method for assessing intake in large groups, including population-based investigation^{16,74}. Food frequency questionnaires typically consist of questions examining the individual's diet quality and quantity⁸³. Questions and foods included in food frequency questionnaires can vary based on the population being studied⁷⁴. Factors that should be considered when developing food frequency questionnaires include age, ethnicity, culture, and the intent of the study or investigation⁸⁴. General food frequency questionnaires include lists of foods and beverages, and individuals are asked to indicate the frequency of consumption of those foods and beverages listed⁸³. Food frequency questionnaires can be distributed and completed in various outlets—they can be mailed to individuals for completion¹⁶, completed by individuals in person, or completed through interviews with trained personnel^{74,75}. Strengths of this method include low cost, ease of use and relative dissemination to large groups^{16,74}. Food frequency questionnaires, however, can be problematic, as they are usually not standardized and rely on the ability of the individual to recall food intake over a specified interval in the past^{74,75}.

Andersen and colleagues explored food frequency questionnaires in assessing energy intake in infants aged twelve months¹⁶. Individuals were provided with a booklet with photographs to help with portion size estimation. Food frequency questionnaires overestimated energy intake by 25% as compared to directly weighed foods¹⁶. Marriott and colleagues investigated the use of interview administered food frequency questionnaires as compared to four day directly weighed foods records to assess energy intake in infants at six months and at twelve months of age^{74,75}. In these studies, trained personnel completed interview administered food frequency questionnaires to parents and caregivers of children enrolled in the study. In infants aged six months, the food frequency questionnaire (Mean=3329 kJ) overestimated energy intake

by 6.2% compared to the directly weighed foods method (Mean= 2968 kJ). Throughout the interview, individuals were asked to describe portion sizes using household measures and food models⁷⁵. In this study, the relatively small error of the food frequency questionnaire as compared to the directly weighed foods may be explained because it was administered by interview rather than directly completed by the parents or caregivers⁷⁵. Food frequency questionnaires have been shown to overestimate energy and specific nutrient intake, but they are useful in estimating average energy intake and dietary patterns on a population level¹⁶.

Remote Food Photography Method

Overview

Methods for quantifying food intake by self-report or weighing have several disadvantages that have directed researchers to develop alternative techniques with lower levels of burden without sacrificing accuracy⁹. Methods reliant on self-report have the largest magnitude of error resulting from the inability of individuals to accurately estimate portion size even after portion estimation training^{7,85}. Methods reliant on direct weighing of foods or infants are extremely burdensome and therefore have a high rate of attrition. Since almost half of Americans already own a smartphone^{22,86}, it appears that effective food intake assessment methods may incorporate the use of advancing technology and smartphones⁹. There are several web-based programs and smartphone applications that allow users to enter food information to estimate energy and macronutrient intake. These methods still ultimately rely on self-report and the data are only as good as the user but are becoming increasingly popular since the need for a dietitian is removed. Recently, digital photography of foods has been utilized to quantify food intake in clinical settings. Digital photography has the advantage to improve portion size estimation in comparison to subjective methods⁹. Recent evidence suggests that adopting digital photography of foods into an assessment method may be a useful tool in free-living conditions⁷.

The Remote Food Photography Method (RFPM), developed by investigators at Pennington Biomedical Research Center (PBRC), is a novel method that can estimate energy and nutrient intake from digital photographs captured of food provision and plate waste^{7,21}. The RFPM is a semi-automated method, namely data collection (photographs), data management, and energy and nutrient analysis are automated with human oversight and portion estimation that requires input from a trained dietary professional²². The RFPM requires an individual to take photographs of both food provision (before meal photographs) and plate waste (after meal

photographs)⁷ using the camera-enabled smartphone application, SmartIntake©, also developed at PBRC^{21,22}. Photographs are then transmitted in near real-time over the wireless network to a web-portal application where they are stored and later analyzed in the Food Photography Application© (PBRC, Baton Rouge, Louisiana, United States)²¹. The stored photographs are compared to standard food portions and linked to the foods' nutrient information in order to obtain food gram weights, macronutrient content, and micronutrient content^{7,9,21}.

The RFPM can be used to assess energy and macronutrient intake of a single meal, meals eaten over a twenty-four hour period, or a five to ten day period to represent typical intake. An important and unique feature of SmartIntake© are the message prompts. Ecological momentary assessment (EMA) message prompts have been developed to increase accuracy and data quality when using the RFPM for food intake assessment on multiple days^{7,21}. For example, EMA message prompts are sent to individuals at personalized meal times to remind them to capture images of food selection and plate waste²¹. Example prompts include: "Can you remember to take before and after pictures of your lunch and send them to us?" and "Did you eat or drink anything today and forget to take a picture?"²¹. The use of these EMA message prompts may minimize poor and missing photographs²¹. There are several advantages of the RFPM as compared to subjective methods including reduced patient burden and the elimination of the need for patients to estimate portion size²². Another strength of the RFPM is that the use of EMA message prompts helps to minimize missing data and to promote data quality²¹. The RFPM has the potential to be a useful tool for assessing food intake beyond clinical research settings and into clinical settings²¹.

SmartIntake© Application

The SmartIntake© (Figure 2) application is available for users with iPhone and Android smartphones allowing individuals to use their own smartphones for RFPM data collection and communication with analyzers²². The "easy to use" SmartIntake© application allows individuals to capture before and after photographs of their food and to transmit the photographs for analysis^{21,22}. Individuals are instructed to arrange their food so that all food items are visible and to include a black and white reference card used for sizing vertically in all photographs²². Before food consumption, individuals are asked to capture the "Before Meal" photograph. The SmartIntake© application requires that individuals identify the foods in one of three ways: 1) automatically using bar code scanning, 2) entering a price look up (PLU) codes or 3) adding a voice or text message for food description²². The photograph and any included descriptions are emailed almost immediately via the smartphone through the wireless network to Food

Photography Application©. Individuals enjoy their meal, and then after fifteen minutes have lapsed, an EMA message prompt is received on the smartphone via text messaging prompting the individual to take a second set of photographs of the plate or food container at the end of the meal. Following the capturing of the “After Meal” photographs, the “After Meal” photographs are emailed through the wireless network to the Food Photography Application©. All photographs from the test period are transmitted to the web-portal application for storage and analysis.

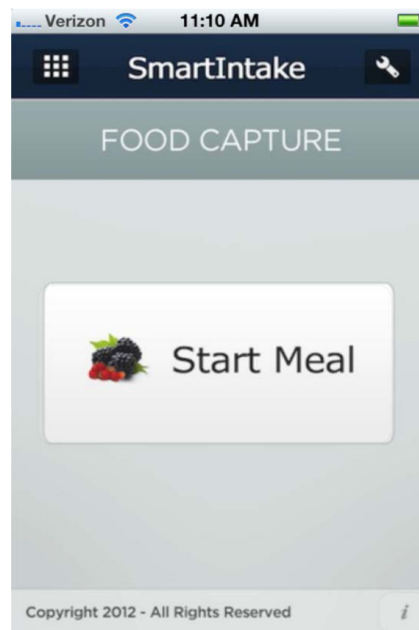


Figure 2: SmartIntake© Application Home Screen

Food Photography Application©

The Food Photography Application© (Figure 3) is a web-portal application responsible for data storage, management, and analysis^{21,22}. Using the digital photography of foods procedures for photo analysis discussed previously⁹, analyzers can estimate photographs for energy and nutrient intake²¹. Standard food portion photographs are housed in the standards database, which provides a standard portion photograph for portion size estimation and a match to nutritional information obtained from the USDA food database, the manufacturer's information, or a custom recipe^{21,22}. Photo analysis requires that USDA codes⁸⁷ for all foods in the photograph be identified as well as the standard photographs and respective serving sizes. Analysis is achieved by the analyzer who provides a ratio of the food portion in the test photograph to the standard food portion^{21,22}. The analyzer opens the test photograph and the appropriate standard

photograph and decides the proportion of the test photograph as compared to the standard photograph to estimate the portion size (Figure 3)^{7,9}. For example, if the test food provision photograph is 1.5 times as large as the standard photograph, the analyzer will input 1.5 as the taken ratio. Then, the analyzer reviews the test plate waste photograph. If the test plate waste photograph is 0.5 times the size of the standard photograph, the analyzer will input 0.5 as the returned ratio. The returned ratio can be subtracted from the taken ratio to provide the consumed ratio. The taken, returned, and consumed ratios are automatically calculated and exported by the Food Photography Application© to provide energy and nutrient information for food intake described in the photographs. Nutrient information gathered from the Food Photography Application© includes gram weights of the food consumed, kilocalories of the food consumed, and gram weights of macronutrients and micronutrients^{7,22}.

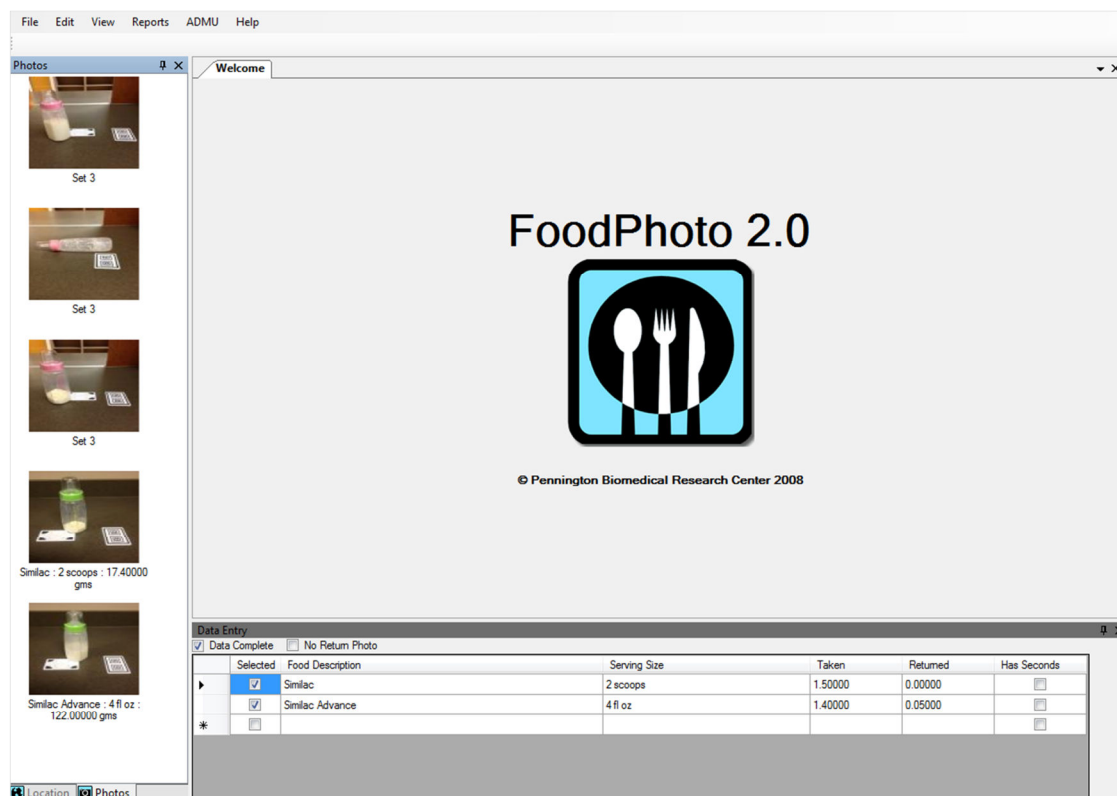


Figure 3: Food Photography Application© Data Estimation

Validation Studies

The digital photography of foods method was the foundation of the portion estimation technology used to assess energy intake in the RFPM⁹. It was validated against directly weighed

foods and was shown to overestimate portion sizes in food selection, plate waste, and food intake as compared to the directly weighed foods method⁹. Figure 4 shows that correlations between digital photography and directly weighed foods for food selection, plate waste, and food intake were high (Range 0.82 to 0.96) for various food types with the exception of condiments (0.63 for food selection, 0.52 for plate waste, and 0.60 for food intake)⁹. A plausible explanation for the difference between digital photography and directly weighed foods for condiments may be that weights of condiments are too small for accurate estimation by a human analyzer⁹.

Table 1
Correlations of estimates of food weights by digital photography and direct visual estimation compared with known weights of food

Food type	Number of items	Food selection		Plate waste		Food intake	
		Direct visual	Digital	Direct visual	Digital	Direct visual	Digital
Overall	453	0.97 ^a	0.94 ^b	0.95 ^a	0.89 ^b	0.96 ^a	0.92 ^b
Entree	60	0.98 ^a	0.87 ^b	0.89	0.91	0.96 ^a	0.85 ^b
Starch	117	0.98 ^a	0.94 ^b	0.95	0.94	0.97 ^a	0.93 ^b
Fruit/vegetable	106	0.96 ^a	0.93 ^b	0.96 ^a	0.89 ^b	0.95 ^a	0.90 ^b
Dessert	60	0.93	0.90	0.92 ^a	0.86 ^b	0.94 ^a	0.82 ^b
Beverage	50	0.93	0.92	0.94	0.96	0.93	0.94
Condiments	60	0.83 ^a	0.63 ^b	0.74 ^a	0.52 ^b	0.81 ^a	0.60 ^b

Note. All correlations were significantly different from 0.00 ($P < .0001$). Different superscripts indicate that the correlations of digital with weighed foods were significantly smaller than the correlations of direct visual with weighed foods ($P < .01$). Note that there were 10 soups that were a part of the overall number of food items that were analyzed. Soups were not analyzed separately because of the small number of observations. "Overall" refers to the average (grams) across all food types.

Figure 4: Correlations of estimates of food weights by digital photography with known food weights (Reprinted from Williamson et al 2003)

Martin and colleagues completed a series of pilot studies to further develop and investigate the uses of the RFPM. The purpose of the first pilot study⁷ was to see if trained analyzers could estimate energy intake from photographs of food provision and plate waste using standard portion photographs. Study procedures consisted of taking photographs of simulated food provision and plate waste in the laboratory and completing directly weighed foods and digital photography. An average underestimation of 8.2% of digital photography as compared to directly weighed foods demonstrated that trained analyzers could adequately estimate energy intake using the digital photography of foods method⁷. The second pilot study⁷ tested if free-living individuals could capture photographs of their food provision and plate waste for several days with trained analyzers using the digital photography of foods similarly to the first pilot study. In addition to capturing photographs, individuals were asked to identify any obstacles to collecting photographs. Conclusions from the second pilot study were that individuals forgot to take photographs occasionally and that review and analysis of photographs could not be completed immediately⁷. Martin and colleagues further developed the RFPM through a validation study against directly weighed foods. In this validation study, foods were prepared in a controlled laboratory and provided to individuals participating in the study. Individuals were instructed to

consume the provided study food items and return waste. Results from analysis of the RFPM in free-living conditions show that RFPM underestimated energy intake by 6.6%⁷.

The RFPM was further investigated using the conclusions and implications from earlier studies. Researchers tested the use of EMA message prompts in the RFPM and the accuracy of the RFPM to estimate energy intake as compared to the DLW. Individuals in the study used smartphones to capture food provision and plate waste photographs over six days in free-living conditions²¹. Study participants received standard or personalized EMA message prompts to remind them to capture photographs. Shown in Figure 5, RFPM with standard EMA message prompts differed significantly from DLW (-895 ± 770 kcal/day, $P < 0.0001$), and RFPM with personalized EMA message prompts did not differ significantly from DLW (-270 ± 748 kcal/day, $P = 0.22$). Moving forward, personalized EMA message prompts were incorporated into the RFPM. The improved RFPM was found to underestimate energy intake by only 3.7% (-152 ± 694 kcal/day, $P = 0.16$) in free-living individuals as compared to DLW (Figure 5)²¹.

Table 2 Comparison of energy and nutrient intake estimates from the Remote Food Photography Method (RFPM) and two gold standards: (i) energy intake (EI) measured by doubly labeled water in free-living conditions, and (ii) energy and nutrient intake measured in laboratory-based buffet meals

	EI measured with the RFPM	EI measured with the gold standard	Difference	P value	Mean participant error (%)
Study 1^a					
Standard Prompts ($n = 22$)	$1,570 \pm 636$	$2,465 \pm 557$	-895 ± 770	<0.0001	-34.3 ± 28.2
Customized Prompts ($n = 13$)	$1,907 \pm 536$	$2,177 \pm 491$	-270 ± 748	0.22	-8.8 ± 29.8
Study 2					
Free-living ($n = 42$) ^a	$2,208 \pm 665$	$2,360 \pm 626$	-152 ± 694	0.16	-3.7 ± 28.7
Buffet Meals ($n = 49$)					
Energy (kcal)	583 ± 190	587 ± 209	-4 ± 73	0.67	1.2 ± 15.1
Fat (kcal)	143 ± 57	136 ± 60	7 ± 19	0.02	8.7 ± 19.8
Carbohydrate (kcal)	350 ± 124	367 ± 136	-17 ± 55	0.04	-3.2 ± 18.3
Protein (kcal)	98 ± 27	92 ± 29	6 ± 17	0.03	10.0 ± 23.9
Vitamin A (mcg)	91 ± 36	78 ± 34	13 ± 17	<0.0001	22.9 ± 31.9
Vitamin C (mg)	7 ± 3	6 ± 2	1 ± 2	0.02	17.3 ± 38.4
Calcium (mg)	246 ± 73	236 ± 73	10 ± 43	0.11	6.9 ± 20.7
Iron (mg)	5 ± 2	5 ± 2	0 ± 1	0.23	3.3 ± 14.6
Sodium (mg)	$1,315 \pm 353$	$1,331 \pm 431$	-15 ± 237	0.65	2.6 ± 20.8
Cholesterol (mg)	48 ± 15	39 ± 14	9 ± 11	<0.0001	30.1 ± 41.2
Fiber (g)	4 ± 1	4 ± 1	0 ± 0	0.95	0.9 ± 13.1

Food intake values reflect mean \pm s.d. P values in bold text were statistically significant (alpha was set at 0.01 for the buffet meal comparisons from the main study and all other comparisons relied on alpha equal to 0.05).

^aDoubly labeled water was used as the gold standard in Study 1 and for free-living comparisons in Study 2. Energy intake in these comparisons reflects kcal/day.

Figure 5: Comparison of energy and nutrient intake estimates by RFPM and the gold standards—energy intake measured by doubly labeled water and laboratory-based buffet meals (Reprinted from Martin et al 2012)

The RFPM has also been investigated in preschool age children. Mothers of preschool age children were trained to use the RFPM to capture photographs of their preschool age children's

foods. Study team members weighed the foods and captured photographs of the foods that the preschool age children consumed in Head Start on the same days. As shown in Figure 6, the mean difference in grams of RFPM as compared to directly weighed foods was 8.8g in food provision, -1.1g in plate waste, and 9.9g in food intake²². These findings showed that the RFPM detects small gram differences, which may be instrumental in using the RFPM in the infant population.



Figure 6 Validity of the Remote Food Photography Method for measuring the food intake (g) of preschool-age children over 24 h in the children's natural environments, including their home and a Head Start (HS) centre.

Figure 6: Mean Gram Difference of RFPM and Directly Weighed Foods in Food Provision, Plate Waste, and Food Intake of Pre-school Children (Reprinted from Martin et al 2013)

CHAPTER 3: MATERIALS AND METHODS

Human Subjects Protection

The Baby Bottle study was approved by the PBRC Institutional Review Board (FWA#00006218) on September 26, 2012 (PBRC IRB 12035) and was registered as a clinical trial (NCT01762631) on the United States National Institutes of Health website, www.clinicaltrials.gov. The Baby Bottle protocol, informed consent (Appendix B), and HIPAA authorization (Appendix C) were initially approved by the PBRC IRB on September 26, 2012. An amended Baby Bottle protocol (Appendix A) was approved by the PBRC IRB on November 15, 2012.

Participant Overview

Inclusion criteria:

Participants were included for participation if they were:

- Eighteen years of age or older,
- Willing to complete two study visits about a week apart at PBRC and
- Willing to identify either as a caregiver or non-caregiver.

For this study, a caregiver was defined as an individual who considered himself or herself a parent, grandparent, sibling, aunt or uncle, nanny or babysitter and who provided care to an infant within the last twelve months. A non-caregiver was defined as someone who had not provided care to an infant within the last twelve months.

Exclusion criteria:

Participants were excluded from participation if they were:

- Less than 18 years of age,
- Not willing to complete two study visits about a week apart at PBRC,
- Not willing to identify either as a caregiver or non-caregiver,
- Failure to contact, or
- Failure to report to scheduled study visit.

Participant Recruitment

Interested individuals were recruited and screened through the PBRC Recruitment Core. Individuals were invited to initiate their interest through the PBRC clinical trials website, via advertisements and targeted emails directed to employees within PBRC and residents of the Greater Baton Rouge area. Interested individuals completed an online eligibility survey hosted on the PBRC clinical trials website. Once completed, online eligibility survey results were transmitted to PBRC Recruitment Core staff for initial screening where participant information including name, address, and date of birth was confirmed and a unique subject identification number was issued. After initial screening was completed, potential volunteers were referred to the Reproductive Endocrinology and Women's Health laboratory for study specific screening by the study coordinator. Individuals who satisfied the eligibility criteria were invited to complete the first of two study visits. The Baby Bottle Consort diagram (Figure 7) shows the throughput of study participants in the Baby Bottle study.

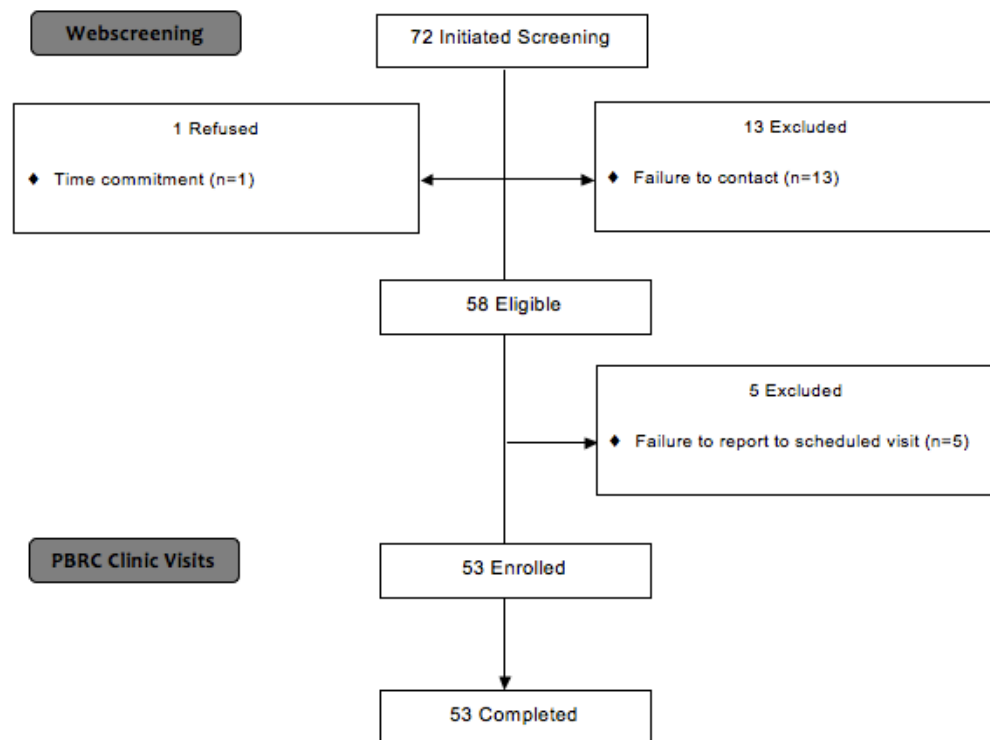


Figure 7: Consort diagram summarizing the throughput of study participants in the Baby Bottle study

Seventy-two individuals completed the eligibility survey to provide 53 participants willing to participate in the study. Of the 72 individuals that initiated screening, 13 (18%) were excluded for failure to contact and 5 (7%) were excluded for failure to report to scheduled visit. One participant (1%) refused participation due to time commitment after study specific screening.

Study Design

The purpose of the Baby Bottle study was to determine the reliability and validity of the RFPM to assess food intake in formula fed infants. The study was comprised of telephone screening and completion of two study visits at PBRC. After signing the Baby Bottle informed consent prior to the start of Visit 1, participants completed two study visits separated by five to ten days. A schematic of the study procedures is summarized in Table 4.

Table 4: Schedule of Procedures			
	Screening	Testing	
	Telephone	Visit 1	Visit 2
Eligibility Evaluation	X		
Informed Consent and HIPAA		X	
Anthropometry (Height/Weight)		X	
Baby Bottle Questionnaire		X	
RFPM Training		X	
Infant Formula Preparation		X	X
RFPM Testing		X	X
Directly Weighed Foods Method		X	X

Telephone Screening

Following initial screening of potential volunteers by the PBRC Recruitment Core, study specific telephone screening was completed in the Reproductive Endocrinology and Women's Health laboratory by the study coordinator. Potential participants were called for study explanation, and they were invited to participate in the Baby Bottle study. The study coordinator provided an overview of the purpose of the Baby Bottle study, explanation of participant involvement as well as a review of the eligibility criteria. Interested individuals who met eligibility criteria were scheduled for Visit 1 at the conclusion of the telephone screening interview.

Visit 1

Participants completed all Visit 1 procedures at the PBRC Outpatient Clinic and PBRC Ingestive Behavior Laboratory. Visit 1 was a one-on-one visit between the participant and the study coordinator. This was a non-fasting visit. Upon arrival, participants were provided the informed consent form (Appendix B) and HIPAA authorization (Appendix C) to read and review at their own pace. After all questions and concerns were addressed and prior to study procedures being conducted, interested participants provided informed consent. Non-fasting body weight and height were measured in the PBRC Outpatient Clinic, and body mass index (BMI) was calculated. Participants were asked about concurrent medications in line with standard PBRC practices, which were documented on the PBRC Concurrent Medication Datasheet (Appendix G). Participants were asked to complete the Baby Bottle questionnaire developed specifically for this study to ascertain the caregiver status of each subject (Appendix D). Participants were then led to the PBRC Ingestive Behavior Laboratory to complete the remainder of the visit.

Participants completed RFPM training to learn how to capture photos and how to send photos to the study coordinator using the SmartIntake© iPhone application. Each participant prepared bottles of infant formula, in a provided random order, to provide final volumes of 2, 4, 6 and 8 fluid ounces. The procedure included completion of two sets of infant formula preparation; hence, a total of eight bottles were prepared. At this visit, data for each formula preparation was collected using both the RFPM and the directly weighed foods method. Directly measured weights were documented by the study coordinator using the Visit 1 Directly Weighed Foods Datasheet (Appendix E). At the conclusion of Visit 1, participants scheduled Visit 2 for approximately five to ten days later.

Visit 2

Participants returned to the PBRC Ingestive Behavior Laboratory approximately five to ten days after Visit 1 to complete Visit 2. At this visit, participants were instructed to repeat the infant formula preparations according to Visit 1 instructions, though they prepared only one bottle of 2, 4, 6, and 8 fluid ounces of infant formula. Each participant prepared the bottles, in a random order, providing 2, 4, 6, and 8 fluid ounces of infant formula. All infant formula preparation and photo capturing procedures were identical to the Visit 1 procedures. Data for each formula preparation was collected using both the RFPM and the directly weighed foods method. Directly

measured weights were documented by the study coordinator using the Visit 1 Directly Weighed Foods Datasheet (Appendix E).

Participant Compensation

After completion of the Baby Bottle study by each participant, the study coordinator requested study compensation of \$20 for the participant through Louisiana State University according to PBRC standard procedures. Participants were notified when study compensation arrived at PBRC, and study compensation was either picked up by participants or mailed directly to the participants.

Description of Study Procedures

Informed Consenting

The Baby Bottle informed consent form outlined the purpose of the study, what would occur throughout the course of the study, the risks and benefits of the research, and that participation was voluntary. Informed consent was obtained following PBRC standard procedures prior to the start of Visit 1. Participants were also given the Baby Bottle HIPAA authorization, which is an extension of informed consenting. The HIPAA authorization outlines that all personal information is kept confidential and secure, and that all published data is de-identified. Participants were given the Baby Bottle informed consent form and HIPAA authorization to read and review with ample time. In some cases, the informed consent form and HIPAA authorization were emailed to participants prior to Visit 1. All questions were answered, and participants verbalized understanding of study procedures prior to signing the informed consent. Each participant signed the informed consent form and HIPAA authorization, and signed copies were provided to participants.

Anthropometrics

Non-fasting body weight and height were measured in duplicate at Visit 1 on all participants according to PBRC standard procedures to calculate BMI values. Non-fasting body weight was measured in light clothing to the nearest 0.1 kg on a calibrated scale (GSE, Livonia, Michigan, United States). Height was measured using a stadiometer (Holtain Limited, Crymych,

United Kingdom) without shoes to the nearest 0.1 cm. The anthropometric measurements were documented by the study coordinator on the PBRC Anthropometric Datasheet (Appendix H) and entered into the PBRC clinical database.

Body Mass Index

Body mass index was calculated using the recorded height and non-fasting body weight at Visit 1 according to PBRC standard procedures. BMI classified each participant as either underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5-24.9 kg/m²), overweight (BMI 25.0-29.9 kg/m²), or obese (BMI ≥ 30 kg/m²)⁸⁸. BMI was calculated and documented by the study coordinator on the PBRC Anthropometric Datasheet (Appendix H) and entered into the PBRC clinical database.

Chart Reporting

Each participant's study documents were maintained in a study chart. All study documents (checklists, source documents, questionnaires, etc.) were reviewed prior to the end of each visit. Written documentation was completed following standard PBRC chart reporting procedures. Study charts were secured in PBRC Medical Records at all times with the exception of study visits. Throughout the study and when participants completed participation, charts were reviewed for incomplete and inconsistent data.

Study-Specific Questionnaire

The Baby Bottle Questionnaire (Appendix D) was provided to participants to collect information on demographics and caregiver status. The questionnaire included demographic questions regarding age, gender, race, smoking history, education, household income, and employment status. Caregiver status questions included if the participant was a parent or guardian, if the participant had children and their ages, if the participant had cared for an infant within the last year, and if the participant had prepared an infant formula bottle within the last year. The participant's classification as a non-caregiver or caregiver was determined through self-report from his or her answer to Question 5 on the Baby Bottle Questionnaire.

Infant Formula Preparation

Given the expense associated with preparing the 636 bottles of infant formula required for this study, whole dry milk was used as a cost effective substitute for commercial powdered infant formula. The whole dry milk powder was transferred to a commercially available, Similac Advance (Abbott Laboratories, Abbott Park, Illinois, United States) infant formula container. Participants were instructed to prepare the infant formula according to the instructions on the container using the provided infant formula scoop. Similac Advance instructions (Figure 8) state: one unpacked level scoop of powdered formula (8.7g) yields a 2 fluid ounce bottle of infant formula, two unpacked level scoops of powdered formula (17.4g) yield a 4 fluid ounce bottle, three unpacked level scoops of powdered formula (26.1g) yield a 6 fluid ounce bottle, and four unpacked level scoops of powdered formula (34.8g) yield an 8 fluid ounce bottle.



Figure 8: Similac Advance Mixing Guide (Located on Infant Formula Container)

To improve infant formula preparation, an RFPM infant formula standard card was developed prior to the initiation of study procedures. Since powdered dry formula is the source of kilocalories of prepared infant formula, it is crucial to estimate dry food provision accurately. The RFPM infant formula standard card (Figure 9) has a space for 1, 2, 3, and 4 scoops of powdered dry formula on the front, and the back of the card includes a space for 0.5, 1.5, 2.5, and 3.5 scoops of powdered dry formula. By placing the infant formula bottle on the space with the appropriate number of scoops, the individual can report the number of scoops of powdered dry formula contained in the prepared infant formula bottle. In analysis of the infant formula photographs, the analyzer has an advantage of using the reported number of scoops in estimation. This will increase the chance of adequate approximation of caloric content.

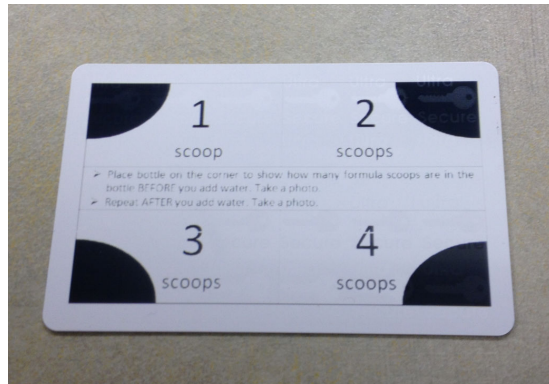


Figure 9: RFPM Infant Formula Standard Card

Prior to beginning the first bottle preparation, participants were trained on the order in which to complete each step of infant formula preparation and photo capturing. Participants were trained on how to capture and send photos using the SmartIntake© iPhone application. The manner in which participants were to prepare infant formula was not demonstrated during training to maintain ecological validity and generalizability of the results. Participants were encouraged to read the instructions for infant formula preparation provided by the manufacturer on the infant formula container. This allowed participants to interpret instructions and prepare the infant formula bottles similarly to how they would prepare the bottles in a free-living situation.

For the RFPM and directly weighed foods method, study procedures required a commercially available infant formula container, empty infant formula bottles, an iPhone with the SmartIntake© application, and the two RFPM standard cards. For each bottle preparation, the participant used the formula scoop provided to measure the required amount of powdered formula necessary to prepare the designated serving size. The participant dispensed the designated number of scoops of powdered formula into the formula bottle. After the powdered formula was dispensed into the clear formula bottle, the participant captured a photo of the formula bottle. This was referred to as *dry food provision* in the RFPM. The formula bottle containing the powdered formula was then weighed on a scale by the study coordinator and the weight was recorded. Weights (to the nearest 0.1 g) were measured using a Mettler Toledo PB3001 scale (Columbus, Ohio, United States). The participant was then instructed to add the desired amount of water to the bottle and mix the contents by vigorous shaking. Using the SmartIntake© application, the participant captured a photo of the prepared formula bottle. This was called *liquid food provision* in the RFPM. The prepared formula bottle was weighed on the scale by the study coordinator, and the weight was recorded.

To simulate infant food intake, the study coordinator discarded a predetermined portion of the prepared formula and the remaining formula bottle was weighed. The volume of formula

discarded was determined randomly between 25-100% according to a Gaussian distribution and a random number generator (Mean=0.80, SD=0.20). Using the SmartIntake© application, the participant captured a photo of the formula bottle after the designated random portion of the prepared formula had been discarded. This was referred to as *liquid waste*. The amount of infant formula remaining as waste was not disclosed to participants. The captured photos from the SmartIntake© application were used to determine if the RFPM accurately estimated simulated infant food intake, which was calculated as food provision minus waste.

Demonstration of capturing photos using the RFPM and the directly weighed foods method is illustrated in Figure 10. Figure 10A shows the dry food provision photo taken by the participant using the SmartIntake© application. After the participant captured the dry food provision photograph, the study coordinator used the directly weighed foods method to weigh the dry food provision (Figure 10B). The bottle was returned to the participant to prepare the infant formula and Figure 10C is the liquid food provision photograph taken by the participant. After the participant captured the liquid food provision photograph, the study coordinator weighed liquid food provision as shown in Figure 10D. The prepared formula bottle was weighed again after discarding the appropriate random amount to represent the bottle waste (Figure 10E). Figure 10F shows the waste photo taken by the participant using the SmartIntake© application.

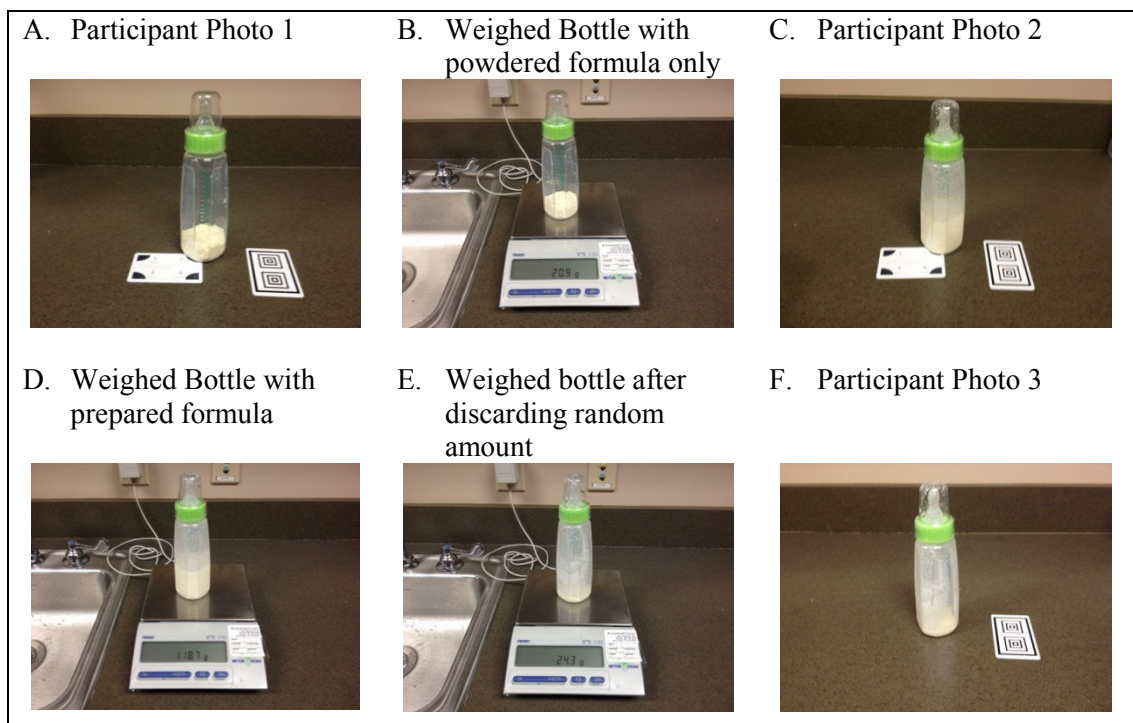


Figure 10: Demonstration of the RFPM and the Directly Weighed Foods method

Statistical Considerations

The primary aim of the Baby Bottle study was to assess if the RFPM can accurately estimate simulated food intake compared to the gold standard, the directly weighed foods method. Power calculations and sample size estimates were performed on the main outcome variable, simulated food intake. Two measures of food provision were obtained: dry food provision (powdered formula) and liquid food provision (prepared formula), the latter is created by adding water to the powdered formula. Food waste is defined as the prepared liquid formula remaining in a bottle after simulating feeding. The study coordinator achieved feeding simulation by discarding at least 25% and at most 100% of the liquid food provision. Food intake was calculated as food provision minus food waste. A secondary aim was to evaluate the inter- and intra- individual variability in infant formula preparation; hence, this study was also appropriately powered to detect differences in the grams of dry powdered formula in the bottle.

Sample Size Estimate

A power analysis was conducted for the Bland-Altman procedure⁸⁹ that was used to determine if the RFPM significantly overestimated or underestimated food provision, food waste, or food intake and if the error associated with the RFPM varied over the amount of food provision, food waste, and food intake. Determining if error variance differs over levels of food intake is critical to examining validity and accuracy. Therefore, the sample size was established based on the regression analysis used to do so since it required the largest number of participants. Power for measuring differences in food intake (liquid food provision minus liquid waste) between the two methods was calculated with variance estimates for intake of beverages from previous studies of the RFPM (Table 5)⁹.

Table 5: Effect Size Calculations for Food Intake (Provision minus Waste)				
n per group	Minimum detectable difference in formula weight (g)	SD (g)	Power	Effect Size
30	6.80	9.71	0.80	0.70
40	5.83	9.71	0.80	0.60
45	5.44	9.71	0.80	0.56
50	5.15	9.71	0.80	0.53
53	4.95	9.71	0.80	0.51
55	4.86	9.71	0.80	0.50
60	4.66	9.71	0.80	0.48

The power analysis indicated that an R^2 of 0.14 could be detected with 53 participants (Power=0.80) which was considered acceptable based on studies that used Bland-Altman analysis on biological parameters⁹⁰. Research indicates that poor measures frequently have $R^2 \geq 0.16$; therefore, a sample size of 53 participants yielded adequate statistical power in the analyses for the primary aim. As illustrated in Table 5, with 53 participants and the acknowledged statistical assumptions, there was power of 0.80 to detect a 4.95 g difference between RFPM estimates and directly weighed food weights of liquid food provision.

The observed power was identified for determining the difference between the dry food provision between RFPM and the directly weighed foods method (Table 6). As shown in Table 6, with 53 subjects, a difference of 2.57g (Effect Size=0.55) could be detected. This power analysis relied on variance estimates for condiments from previous studies of the RFPM⁹, which are similar to infant formula. This effect size of 2.57g reflected a very small amount of dry powdered formula, indicating that the study was also sufficiently powered to measure differences in dry food provision. The proposed sample size and data analysis plan represented a viable alternative to equivalence tests that require large sample sizes.

Table 6: Effect Size Calculations for Dry Food Provision				
n per group	Minimum detectable difference in formula weight (g)	SD (g)	Power	Effect Size
30	3.51	4.68	0.80	0.75
40	3.00	4.68	0.80	0.64
45	2.81	4.68	0.80	0.60
50	2.62	4.68	0.80	0.56
53	2.57	4.68	0.80	0.55
55	2.53	4.68	0.80	0.54
60	2.39	4.68	0.80	0.51

Randomization

Randomization was employed for the infant formula preparation procedures, namely the order participants prepared different sized infant formula bottles of 2, 4, 6 and 8 fluid ounces. Randomization was necessary to evenly distribute a potential learned effect across preparations. Participants received three set assignments for the order in which the different sized infant formula bottles were to be prepared with an example shown in Table 7.

Table 7: Example Randomization Schedule for Infant Formula Preparation					
		Bottle 1	Bottle 2	Bottle 3	Bottle 4
Visit 1	Set 1	2 fluid oz	4 fluid oz	8 fluid oz	6 fluid oz
	Set 2	6 fluid oz	4 fluid oz	2 fluid oz	8 fluid oz
Visit 2	Set 3	4 fluid oz	8 fluid oz	6 fluid oz	2 fluid oz

The randomization plan for discarding prepared infant formula was determined using a random number generator and reflected a Gaussian distribution (Mean=0.80, SD=0.20). All numbers over 100% that were generated were assumed to be 100%. A sample of the random numbers generated for discarding prepared infant formula is shown in Table 8.

Table 8: Example Randomization for Discarding Prepared Infant Formula					
		Bottle 1	Bottle 2	Bottle 3	Bottle 4
Visit 1	Set 1	100%	75%	60%	100%
	Set 2	100%	67%	74%	80%
Visit 2	Set 3	68%	57%	75%	64%

Remote Food Photography Method Analysis

Food Photography Application© Training

Before testing the validity of the RFPM method against the directly weighed foods method, the study coordinator was trained to use the Food Photography Application© analysis application. The Food Photography Application© is a computer program that is used to manage data and analyze images of foods using existing and validation visual comparison methods^{7,9,21}. The study coordinator was trained by the master rater in the Food Photography Application©. The Food Photography Application© training procedure included three phases. Phase 1 was an interactive practice phase in which the master rater and the trainee rater analyzed five sample sets of photographs. This allowed the master rater to show the trainee rater examples and to answer questions throughout the practice phase. Phase 2 and Phase 3 utilized a sample size of 25 sets of photographs to allow for the detection of 5% error between the two raters. In Phase 2, each rater analyzed the photos independently. Bland-Altman regression plots and dependent t-tests were generated to test if the two sets of ratings were significantly different, and the intra-class correlation coefficient (ICC) was calculated to examine inter-rater agreement. In previous studies,

an ICC of 0.95 was considered acceptable to show inter-rater agreement^{7,9}; hence, this criterion was used in this study. Phase 2 was repeated in its entirety as Phase 3 to demonstrate an improvement in the accuracy of the trainee's estimates, or a training effect. The goal of the training exercises was to achieve within 5% error between the trainee rater and the master rater to show that the trainee rater was adequately trained to analyze photographs through the Food Photography Application©, and that the trainee rater could analyze the Baby Bottle study photographs with confidence. The Food Photography Application© training procedure did not address the accuracy or validity of the Food Photography Application© and the RFPM in estimating simulated infant food intake as compared to the gold standard, the directly weighed foods method, as this was the purpose of the Baby Bottle study.

Justification of Photograph Sets Needed to Assess Accuracy and Precision in Food Photography Application© Training

To determine the minimum sample size (set of photographs) needed to detect a 5% difference between the master rater and the trainee rater, a pilot set of formula bottles were prepared (in random order) and used to calculate the mean and standard deviation for measured dry and liquid formula preparations for of 2, 4, 6, and 8 fluid ounce bottles (Table 9). The minimum sample size needed to detect differences between raters was determined using the mean and standard deviation of the measured dry formula (Table 9) for each feeding size, $\beta=0.8$, $\alpha=0.05$, and percent error between raters of 5% to 10%. Sample size estimates for each bottle preparation are summarized in Table 10. The minimum sample size (set of photographs) needed to detect a 5% difference between raters for a 2 fluid ounce preparation of infant formula was 21 (Table 10). A sample size of at least 21 dry photos allowed for a 5% error to be detected between the trainee rater and the master rater. Hence, the following sets of photographs were used to quantify inter-rater agreement: 25 preparations of dry formula photographs, 25 preparations of liquid formula photographs, and 25 preparations of liquid waste photographs.

Table 9: Mean and Standard Deviation for Measurement of Dry Formula						
Bottle Size (oz)	Dry Weight (g)	Wet Weight (g)	Dry Mean (g)	Dry SD (g)	Wet Mean (g)	Wet SD (g)
2	8.1	65.8				
2	9.6	68.2				
2	9.3	65.1				
2	8.6	67				
2	9.9	68	9.1	0.738	66.8	1.354

Table 9 Continued: Mean and Standard Deviation for Measurement of Dry Formula						
Bottle Size (oz)	Dry Weight (g)	Wet Weight (g)	Dry Mean (g)	Dry SD (g)	Wet Mean (g)	Wet SD (g)
4	18.5	134.2				
4	20.2	139.4				
4	19.7	138.1				
4	18.1	133.3				
4	18.9	133.5	19.1	0.861	135.7	2.841
6	27.3	201.3				
6	28.6	202.4				
6	28.8	202.5				
6	28.1	206.3				
6	29.4	205.5	28.4	0.789	203.6	2.170
8	37.6	269.9				
8	37.8	270.5				
8	37.4	273.8				
8	38.7	273.4				
8	34.9	271.6	37.3	1.420	271.8	1.724

Table 10: Sample Size Calculations for 5% Measurement Error of Infant Formula						
Bottle Size (oz)	Expected weight (g)	Estimated weight (g)	Measurement error (%)	SD expected weight (g)	Effect Size	Sample Size
2	9.1	8.645	5	0.738	0.617	21
2		8.372	8		0.986	17
2		8.19	10		1.233	11
4	19.1	18.145	5	0.861	1.109	13
4		17.572	8		1.775	5
4		17.19	10		2.218	4
6	28.4	26.98	5	0.789	1.800	5
6		26.128	8		2.880	2
6		25.56	10		3.599	2
8	37.3	35.435	5	1.42	1.313	10
8		34.316	8		2.101	4
8		33.57	10		2.627	3

Food Photography Application© Baby Bottle Analysis

During the Baby Bottle study, participants captured three photographs per bottle with the SmartIntake© application. After collection of photographs through the SmartIntake© application, photographs were analyzed using the Food Photography Application© as described previously in

Chapter 2. Two scoops of Similac Advance (17.4 g) and four fluid ounces of Similac Advance (122 g) were arbitrarily chosen as USDA database⁸⁷ standards in this analysis. The first photograph, dry food provision, was analyzed against a two-scoop standard of Similac Advance. The second and third photographs, liquid food provision and waste, were analyzed against a four fluid ounce Similac Advance standard bottle. Analysis of the photographs by the study coordinator provided an estimate of the proportion of the standard portion that was present in the images of participants' bottles.

Data Analysis Plan

Demographic and Descriptive Analysis

Demographic and descriptive statistics of the study population were generated. Continuous variables including age and BMI were expressed as means and standard deviations. Categorical variables including race, gender, and BMI group were expressed as percentages. Student's t-tests generated P values using Microsoft Excel 2010 (Microsoft, Redmond, Washington, United States). Statistical significance was set at alpha equal to 0.05.

Primary Analysis

Paired dependent t-tests were conducted to determine if the RFPM estimates differed significantly from the directly weighed food weights, and the Bland-Altman regression method⁸⁹ was employed to determine if error variance differed over the amount of food provision and intake. Bland-Altman regression plots⁸⁹ were generated using Sigma Plot 12.0 (Systat Software, San Jose, California, United States). Dry food provision, liquid food provision, and liquid intake were compared using paired dependent t-tests and the Bland-Altman regression method⁸⁹. Statistical significance was set at alpha equal to 0.05.

Secondary Analysis

Multivariate analysis of variance (MANOVA) was used to analyze the secondary objectives of the Baby Bottle study. Secondary objectives were to evaluate the inter- and intra-individual variability in infant formula preparation and to investigate the variability in infant formula preparation between caregivers and non-caregivers of infants. Both dry food provision

and liquid food provision were evaluated as dependent variables. Trial number (1, 2, 3), otherwise known as time, and caregiver status (caregiver or non-caregiver) were treated as construct model effects for both dependent variables to analyze the effects of time and caregiver status on infant formula preparation. Statistical analyses were performed using JMP version 10.0.0 (SAS Institute, Cary, North Carolina, United States). Statistical significance was set at alpha equal to 0.05.

CHAPTER 4: RESULTS

Food Photography Application© Training

Inter-Rater Analysis: Dry Food Provision

Twenty-five dry formula photographs were analyzed in Phase 2 Training and Phase 3 Training in the Food Photography Application© independently by both raters. The Food Photography Application© analysis provided before, after, and consumed gram weights. For dry formula photograph analysis, all “after” gram weights were set at zero, so “before” gram weights were equaled to the consumed gram weights. Consumed gram weights were utilized in the analysis.

In Phase 2 Training, the mean gram weights of the two raters did not significantly differ ($P=0.2985$, Figure 11A, B). An ICC of 0.9499 suggested strong agreement between the raters. As indicated in Table 11, average percent error with respect to the master rater was -11.765% (Range -28.571, 0.000) in one scoop bottles, 1.493% (Range 0.000, 4.348) in two scoop bottles, 11.005% (Range -2.857, 30.769) in three scoop bottles, and 0% (Range -16.000, 16.667) in four scoop bottles. The Bland-Altman regression plot (Figure 11B) showed no significant trend, indicating that bias did not differ by the amount of dry powdered formula in the bottle ($y = -0.3671x + 24.94$, $R^2 = 0.0122$, $P = 0.5984$).

Table 11: Average Percent Error of Trainee Rater With Respect to Master Rater of Dry Food Provision in Phase 2 Training

	Trainee Rater Average (g)	Master Rater Average (g)	Average Percent Error (%)	Range of Percent Error (%)
2 fl oz (1 scoop)	8.70	9.86	-11.77	-28.57, 0.00
4 fl oz (2 scoops)	19.72	19.43	1.49	0.00, 4.35
6 fl oz (3 scoops)	28.83	25.98	11.01	-2.86, 30.77
8 fl oz (4 scoops)	36.76	36.76	0.00	-16.00, 16.67

In Phase 3 Training, one photograph was a clear outlier and it was removed from the analysis dataset as a rater typing error. Across the 25 sets of photographs, the mean analysis of the trainee rater (24.14 ± 10.33 g) did not differ significantly ($P = 0.6473$) from the mean of the master rater (24.00 ± 10.25 g). (Figure 11C, D). Additionally, an ICC of 0.9976 demonstrated high agreement between the two raters, as the a priori aim was to achieve an ICC of greater than or equal to 0.95. Table 12 shows that the average percent error with respect to the master rater was 0% (Range -16.667, 20.000) for bottles containing one standard scoop of formula, 0%

(Range 0.000, 0.000) for bottles containing two standard scoops of formula, 1.9% (Range -6.667, 14.286) for bottles containing three standards of formula, and 0% (Range -6.667, 14.286) for bottles containing four standard scoops of formula. The Bland-Altman regression plot (Figure 11D) showed no significant trend ($y = -0.3551x + 24.019$, $R^2 = 0.0028$, $P = 0.8056$).

Table 12: Average Percent Error of Trainee Rater With Respect to Master Rater of Dry Food Provision in Phase 3 Training				
	Trainee Rater Average (g)	Master Rater Average (g)	Average Percent Error (%)	Range of Percent Error (%)
2 fl oz (1 scoop)	9.28	9.28	0.00	-16.67, 20.00
4 fl oz (2 scoops)	18.56	18.56	0.00	0.00, 0.00
6 fl oz (3 scoops)	26.85	26.35	1.89	-6.67, 14.29
8 fl oz (4 scoops)	35.02	35.02	0.00	-10.00, 5.00

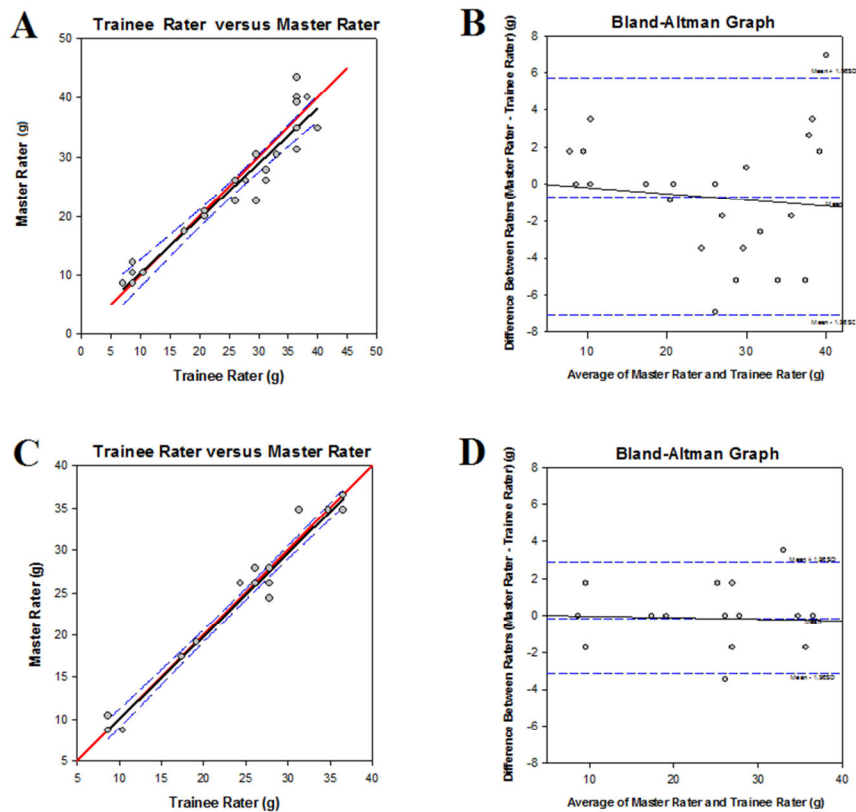


Figure 11: Food Photography Application© Estimates of Dry Food Provision in Phase 2 Training (A, B) and Phase 3 Training (C, D)

Inter-Rater Analysis: Liquid Formula Intake

Fifty liquid formula photographs including 25 “before” and 25 “after” photographs of the same formula bottles were analyzed in Phase 2 Training and Phase 3 Training by both raters in an

independent setting. Analysis of the “before” photographs was used to quantify estimated gram weights. Analysis of the “after” photographs was used to quantify the returned gram weights. The difference between the “before” and “after” gram weights represented the consumed gram weights. Only consumed gram weights were used in analysis.

In Phase 2 Training, the mean of consumed gram weights of the trainee rater (124.90 ± 65.74 g) was significantly different ($P= 0.0269$) from the mean consumed gram weights of the master rater (116.88 ± 70.14 g) (Figure 12A, B). The ICC was 0.8375 and did not achieve the level deemed appropriate for trainer certification of analysis. The average percent error with respect to the master rater (Table 13) was 10.59% (Range 0.000, 27.273) in 2 fl oz bottles, 4.05% (Range -10.000, 14.286) in 4 fl oz bottles, 5.52% (Range -12.727, 29.633) in 6 fl oz bottles, and 8.46% (Range -4.000, 166.667) in 8 fl oz bottles suggesting overestimation of gram weights by the trainee rater as compared to the master rater with an increase in the bottle size. The Bland-Altman regression plot (Figure 12B) showed no significant trend ($y = 1.03x + 129.16$, $R^2= 0.0678$, $P= 0.2088$).

Table 13: Average Percent Error of Trainee Rater With Respect to Master Rater of Liquid Formula Intake in Phase 2 Training

	Trainee Rater Average (g)	Master Rater Average (g)	Average Percent Error (%)	Range of Percent Error (%)
2 fl oz	47.78	43.21	10.59	0.00, 27.27
4 fl oz	78.28	75.23	4.05	-10.00, 14.29
6 fl oz	144.46	137.25	5.25	-12.73, 29.63
8 fl oz	181.93	167.75	8.46	-4.00, 166.67

Given that the comparison between the trainee rater and master rater for liquid formula intake did not reach the pre-determined level ($ICC=0.95$), Phase 3 Training was completed. In Phase 3 Training, the mean liquid formula intake of the trainee rater (121.34 ± 69.36 g) did not significantly differ ($P= 0.7887$) from the mean liquid formula intake of the master rater (120.41 ± 68.58 g) (Figure 12C, D). The calculated ICC was 0.9977 indicating high agreement between the two raters. As shown in Table 14, average percent error of the trainee rater with respect to the master rater was 19.2% (Range 0.000, 40.000) in 2 fl oz bottles, -23.3% (Range -36.842, 12.500) in 4 fl oz bottles, -2.7% (Range -20.000, 16.667) in 6 fl oz bottles, and 3.4% (Range in 0.000, 9.091) 8 fl oz bottles. The Bland-Altman regression plot (Figure 12D) showed no significant trend ($y = -0.18x + 120.71$, $R^2= 0.0021$, $P= 0.8270$).

Table 14: Average Percent Error of Trainee Rater With Respect to Master Rater of Liquid Formula Intake in Phase 3 Training				
	Trainee Rater Average (g)	Master Rater Average (g)	Average Percent Error (%)	Range of Percent Error (%)
2 fl oz	47.28	39.65	19.23	0.00, 40.00
4 fl oz	67.10	87.43	-23.26	-36.84, 12.50
6 fl oz	140.30	144.22	-2.72	-20.00, 16.67
8 fl oz	179.04	173.09	3.44	0.00, 9.09

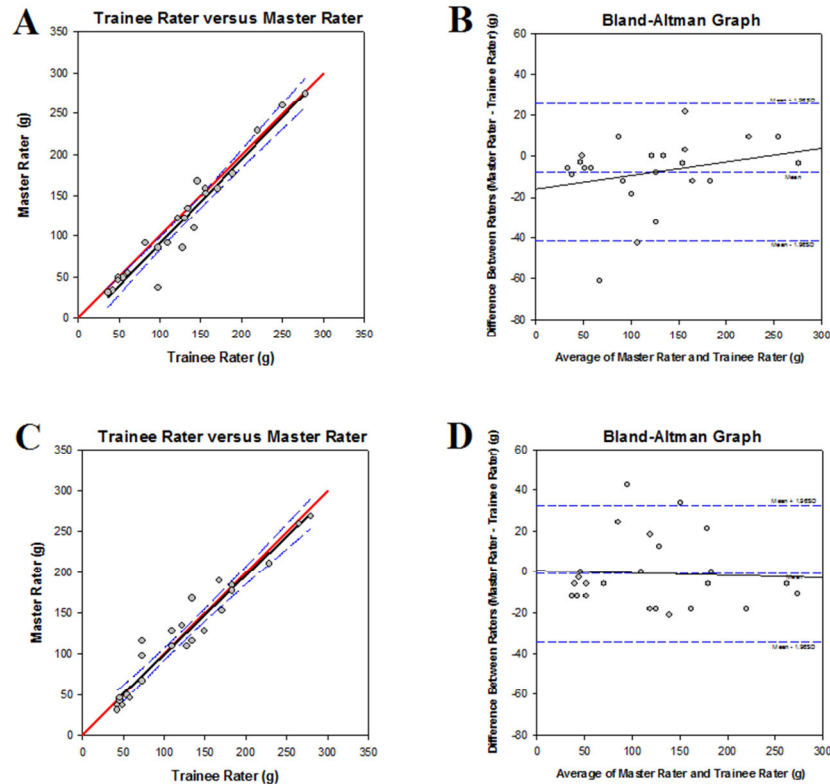


Figure 12: Food Photography Application© Estimates of Liquid Formula Intake in Phase 2 Training (A, B) and Phase 3 Training (C, D)

Demographic and Descriptive Characteristics

The demographic characteristics of the study sample are shown in Table 15. Caregivers and non-caregivers did not differ on the basis of demographic information ($P > 0.05$ for all variables). The average age of participants enrolled in the study was 31 ± 14 years for caregivers and 34 ± 14 years for non-caregivers. Approximately, 89% of caregivers and 88% of non-caregivers in the study sample were female. Sixty-eight percent of caregivers and 80% of non-caregivers in the study sample were Caucasian. According to measured height and weight at Visit 1 and calculated BMI, 54% of caregivers and 68% of non-caregivers in the study sample were

classified as normal weight (BMI 18.5-24.9 kg/m²), 4% of caregivers were classified as underweight (BMI <18.5 kg/m²), 14% of caregivers and 12% of non-caregivers were classified as overweight (BMI 25-29.9 kg/m²) and, 7% of caregivers and 8% of non-caregivers were classified as obese (BMI ≥30 kg/m²).

Table 15: Characteristics of Study Participants			
	Caregivers (n=28)	Non-caregivers (n=25)	P value
Age, y	31 ± 14	34 ± 14	0.43
BMI, kg/m ²	27.1 ± 8.3	24.8 ± 5.4	0.25
Underweight	1 (4)	-	
Normal	15 (54)	17 (68)	
Overweight	4 (14)	3 (12)	
Obese	8 (29)	5 (20)	
Race			0.48
Caucasian	19 (68)	20 (80)	
African American	7 (25)	3 (12)	
Other	2 (7)	2 (8)	
Gender			0.88
Male	3 (11)	3 (12)	
Female	25 (89)	22 (88)	
Continuous variables are expressed as Mean ± SD, Categorical variables are expressed as n (%). BMI= body mass index. Other race includes Asian and no answer.			

Baby Bottle Study- Primary Analysis

Combined

Among all infant formula bottles in the study sample, the RFPM estimated the mean gram weight of dry food provision as 24.1 ± 11.0 g (111.0 ± 50.5 kcals), and the directly weighed foods method estimated the mean gram weight of dry food provision as 22.3 ± 9.9 g (102.5 ± 45.5 kcals). As shown in the scatterplot (Figure 13A), there was high association between the gram weight estimations of the RFPM and the directly weighed foods method ($R^2 = 0.95$, $P < 0.001$). The RFPM significantly underestimated dry food provision ($T = -18.313$, $P < 0.0001$) by a mean of 1.9 ± 2.5 g or 6.7 ± 9.9 % as compared to the directly weighed foods method (Table 16). The Bland-Altman regression plot (Figure 13B) showed a negative trend, indicating that the RFPM had larger underestimates of dry powdered infant formula as the amount of infant formula and bottle size increased ($y = -0.11 (0.01) x + 0.61 (0.22)$, $R^2 = 0.1860$, $P < 0.0001$). The difference between the two methods ranged from an underestimation of 13.74 g to an overestimation of 17.24 g by the RFPM. The mean difference in energy estimation of the RFPM and the directly weighed

foods method was 8.5 ± 11.7 kcal (Table 16). This represents a daily underestimation of 34 to 51 kilocalories based upon recommended feeding schedules of infants (Table 16).

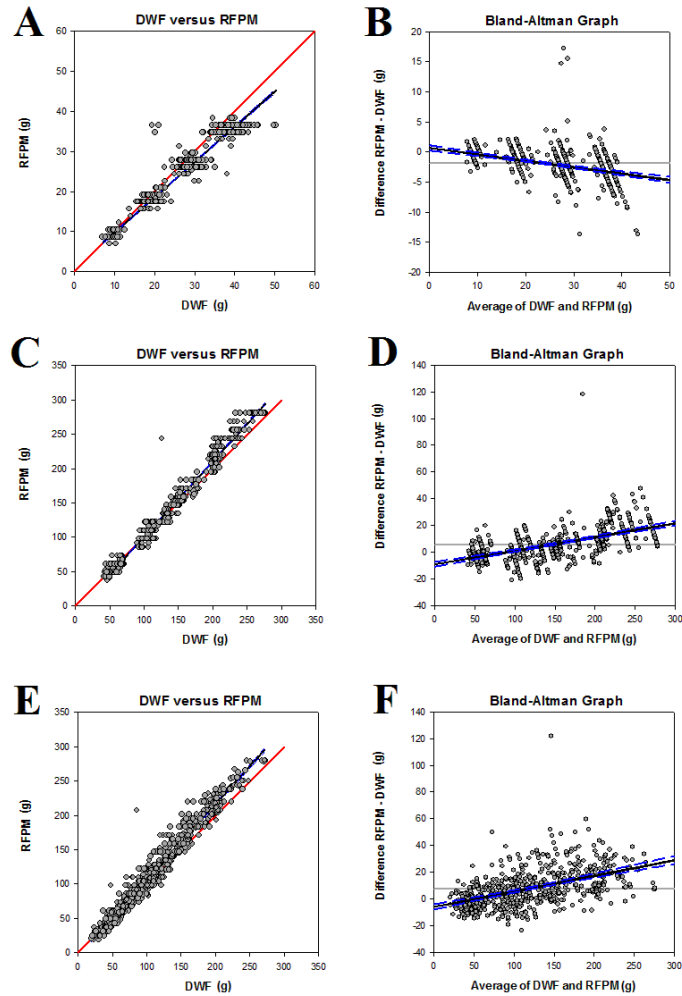


Figure 13: Bland-Altman regression analysis comparing gram weight estimated by the Remote Food Photography Method (RFPM) to the gold standard, Directly Weighed Foods (DWF) in combined sized bottles. The figure shows: (A) Linear regression plot of Dry Food Provision (B) Bland-Altman regression plot of Dry Food Provision (C) Linear regression plot of Liquid Food Provision (D) Bland-Altman regression plot of Liquid Food Provision (E) Linear regression plot of Liquid Intake (F) Bland-Altman regression plot of Liquid Intake

After preparing liquid food provision by adding the required amount of water, the mean gram weight estimations shown from the RFPM and the directly weighed foods were 149.0 ± 73.4 g (102.5 ± 45.5 kcals) and 143.4 ± 66.2 g (111.0 ± 50.5 kcals), respectively (Table 16). As shown in the scatterplot (Figure 13C), gram estimations by the RFPM and the directly weighed foods were strongly associated ($R^2 = 0.98$, $P < 0.001$). The RFPM significantly overestimated

liquid food provision among all prepared liquid formula bottles ($T= 11.7017$, $P<0.0001$) by 5.7 ± 12.2 g as compared to the directly weighed foods method (Table 16). This resulted in a mean percent difference of $2.5 \pm 9.0\%$ between methods (Table 16). The Bland-Altman regression plot (Figure 13D) showed a significant positive trend, indicating that the RFPM underestimated the amount of infant formula intake with smaller bottle sizes and overestimated the amount of infant formula intake with larger bottle sizes ($y= 0.10 (0.01) x - 9.35 (0.91)$, $R^2=0.3430$, $P<0.0001$). The difference between the two methods ranged from an underestimation of 21.10 g to an overestimation of 47.50 g by the RFPM, excluding the outlier that differed between methods by 118 g. Since dry infant formula powder accounts for energy content of the prepared liquid formula, the mean difference in energy estimation between the RFPM and the directly weighed foods method among all prepared liquid formula bottles was the same as with the dry food provision, 8.5 ± 11.7 kcals resulting in a daily underestimation of the RFPM of 34 to 51 kilocalories (Table 16).

The mean estimation of liquid intake among all infant formula bottles using the RFPM was 117.9 ± 65.6 g (81.1 ± 40.7 kcal) (Table 16), and the mean estimation of liquid intake among all infant formula bottles using the directly weighed foods method was 110.8 ± 58.4 g (85.8 ± 44.5 kcal) (Table 16). As shown in the scatterplot (Figure 13E), there was a strong association between the gram weight estimations of the RFPM and the directly weighed foods method ($R^2= 0.96$, $P<0.0001$). The RFPM significantly underestimated liquid intake by 7.2 ± 14.1 g as compared to the directly weighed foods method ($T= 12.8241$, $P <0.0001$) (Table 16). This is also represented as a mean percent difference between methods of $4.9 \pm 15.0\%$ (Table 16). The Bland-Altman regression plot (Figure 13F) that compared mean estimations of the methods and the differences between methods of liquid intake showed a significant positive trend ($y= 0.12 (0.01) x - 6.22 (1.01)$, $R^2=0.2626$, $P<0.0001$). The difference between the two methods ranged from an underestimation of 23.70 g to an overestimation of 59.60 g by the RFPM, excluding the outlier that differed between methods by 121.80 g. The mean difference of energy estimation of the liquid intake in 2 fluid ounce bottles was 4.6 ± 9.5 kcal providing a daily underestimation of liquid intake of 18 to 27 kcals by the RFPM as compared to the directly weighed foods method (Table 16).

Table 16: Comparison of Measurement of Infant Formula Intake by DWF and RFPM

		Weight Estimation (g)				Energy Estimation ⁴ (kcal)					
Infant Formula Preparations		DWF	RFPM	P value ¹	Mean Gram Difference ²	Percent Error Estimate of Gram Weight ³	Error Estimate of Energy Intake per Formula Preparation (kcal)	Error Estimate of Energy Intake per Day ⁵ (kcal)			
2 oz											
Dry Provision		9.8 ± 1.1	9.2 ± 1.0	<0.0001	-0.6 ± 1.0	-5.7 ± 10.2	44.8 ± 4.7	42.1 ± 4.8	<0.0001	-2.8 ± 4.6	11.1, 16.6
Liquid Provision		57.6 ± 7.8	56.2 ± 7.6	0.0024	-1.4 ± 5.8	-1.9 ± 10.3	44.8 ± 4.7	42.1 ± 4.8	<0.0001	-2.8 ± 4.6	11.1, 16.6
Liquid Intake		45.9 ± 10.5	43.8 ± 10.9	<0.0001	-2.2 ± 6.2	-4.1 ± 14.4	35.7 ± 6.3	32.7 ± 6.9	<0.0001	-3.0 ± 4.4	11.8, 17.8
4 oz											
Dry Provision		19.3 ± 1.8	18.1 ± 2.7	<0.0001	-1.1 ± 2.8	-5.5 ± 13.9	88.6 ± 8.1	83.4 ± 12.5	<0.0001	-5.2 ± 12.9	20.8, 31.2
Liquid Provision		115.1 ± 16.3	115.7 ± 19.1	0.5224	0.7 ± 12.8	0.9 ± 10.9	88.6 ± 8.1	83.4 ± 12.5	<0.0001	-5.2 ± 12.9	20.8, 31.2
Liquid Intake		90.1 ± 23.9	91.5 ± 24.8	0.1550	1.5 ± 13.1	2.8 ± 16.3	69.3 ± 11.8	65.9 ± 16.3	<0.0001	-3.6 ± 10.5	14.5, 21.8
6 oz											
Dry Provision		28.9 ± 2.1	26.5 ± 1.1	<0.0001	-2.4 ± 2.2	-7.9 ± 6.9	132.9 ± 9.7	121.9 ± 5.2	<0.0001	-11.0 ± 10.1	44.2, 66.2
Liquid Provision		174.1 ± 24.2	180.0 ± 27.6	<0.0001	5.9 ± 8.6	3.3 ± 4.9	132.9 ± 9.7	121.9 ± 5.2	<0.0001	-11.0 ± 10.1	44.2, 66.2
Liquid Intake		133.6 ± 40.7	141.8 ± 42.2	<0.0001	8.2 ± 10.3	7.0 ± 12.4	102.0 ± 16.3	96.0 ± 7.9	<0.0001	-5.9 ± 9.1	23.6, 35.5
8 oz											
Dry Provision		38.6 ± 3.0	35.4 ± 1.2	<0.0001	-3.3 ± 2.8	-7.9 ± 6.7	177.6 ± 13.9	162.7 ± 5.5	<0.0001	-14.9 ± 12.9	59.7, 89.6
Liquid Provision		226.6 ± 24.2	244.1 ± 24.4	<0.0001	17.5 ± 10.5	7.9 ± 4.9	177.6 ± 13.9	162.7 ± 5.5	<0.0001	-14.9 ± 12.9	59.7, 89.6
Liquid Intake		173.5 ± 47.4	194.6 ± 45.5	<0.0001	21.2 ± 12.9	14.0 ± 10.3	136.0 ± 27.2	129.7 ± 10.3	<0.0001	-5.7 ± 12.0	23.0, 34.4
Combined											
Dry Provision		24.1 ± 11.0	22.3 ± 9.9	<0.0001	-1.9 ± 2.5	-6.7 ± 9.9	111.0 ± 50.5	102.5 ± 45.5	<0.0001	-8.5 ± 11.7	34.0, 50.9
Liquid Provision		143.4 ± 66.2	149.0 ± 73.4	<0.0001	5.7 ± 12.2	2.5 ± 9.0	111.0 ± 50.5	102.5 ± 45.5	<0.0001	-8.5 ± 11.7	34.0, 50.9
Liquid Intake		110.8 ± 58.4	117.9 ± 65.6	<0.0001	7.2 ± 14.1	4.9 ± 15.0	85.8 ± 44.5	81.1 ± 40.7	<0.0001	-4.6 ± 9.5	18.2, 27.4

DWF= Directly Weighed Foods; RFPM= Remote Food Photography Method; All values are expressed as Mean ± SD.

¹P values were calculated using Paired dependent t tests. P values in bold text were statistically significant (alpha was set at 0.05).

²Gram difference (RFPM - DWF) was calculated between paired values, and the mean gram difference among groups is expressed.

³Percent error was calculated from individual data points.

⁴Energy estimations were calculated by multiplying gram weights by kilocalories per gram of Similac Advance (4.5977 kcal/gram)

⁵Error estimates of energy intake per day were calculated for four feedings and six feedings (Recommended feeding range according to the American Academy of Pediatrics²³). Values are expressed as a kilocalorie range.

DWF= Directly Weighed Foods; RFPM= Remote Food Photography Method; All values are expressed as Mean ± SD.

¹P values were calculated using Paired dependent t tests. P values in bold text were statistically significant (alpha was set at 0.05).²Gram difference (RFPM - DWF) was calculated between paired values, and the mean gram difference among groups is expressed.³Percent error was calculated from individual data points.⁴Energy estimations were calculated by multiplying gram weights by kilocalories per gram of Similac Advance (4.5977 kcal/gram)⁵Error estimates of energy intake per day were calculated for four feedings and six feedings (Recommended feeding range according to the American Academy of Pediatrics²³). Values are expressed as a kilocalorie range.

2 fl oz

Using RFPM, a single scoop of infant formula powder (dry food provision), required for preparation of a 2 fluid ounce bottle, was estimated to weigh 9.8 ± 1.1 g (44.8 ± 4.7 kcal) (Table 16). The mean directly measured weight of a single scoop of infant formula powder was 9.2 ± 1.0 g (42.1 ± 4.8 kcal) (Table 16). As shown in the scatterplot (Figure 14A), the RFPM and the directly weighed foods gram weight estimations were weakly associated ($R^2 = 0.28$, $P < 0.0001$). The RFPM significantly underestimated dry food provision ($T = -7.5524$, $P < 0.0001$) by a mean of 0.6 ± 1.0 g or 5.7 ± 10.2 % as compared to the directly weighed foods method (Table 16). The Bland-Altman regression plot (Figure 14B) showed a bias that was not statistically significant ($y = 0.02 (0.09) x - 0.80 (0.84)$, $R^2 = 0.0003$, $P = 0.8152$) with difference between methods ranging from an underestimation of 3.34 g by the RFPM to an overestimation of 2.04 g by the RFPM. The mean difference in energy estimation of the RFPM and the directly weighed foods method was 2.8 ± 4.6 kcal (Table 16). This represents a daily underestimation of 11 to 16 kilocalories based upon recommended feeding schedules of newborns (Table 16).

Additionally, the directly weighed foods gram weights of dry food provision were analyzed to examine the variability of a single scoop of dry powdered formula and the error from the standard size of a single scoop as indicated in the Similac Advance nutrition information. The Similac Advance nutrition information provides that a single scoop of dry powdered formula should weigh 8.7 g. Average size of a single scoop of dry powdered formula showed a mean error of 1.1 g as compared to the standard weight of 8.7 g. Among individuals in the preparation of dry food provision among 2 fluid ounce bottles, error varied from - 0.8 g to 2.3 g as compared to the standard weight of 8.7 g.

After the required amount of water was added to produce liquid food provision, the RFPM and the directly weighed foods method estimated liquid food provision in 2 fluid ounce bottles with mean gram weights of 57.6 ± 7.8 g and 56.2 ± 7.6 g, respectively (Table 16). As shown in the scatterplot (Figure 14C), the RFPM and the directly weighed foods gram weight estimations were moderately associated ($R^2 = 0.52$, $P < 0.0001$). The RFPM significantly underestimated liquid food provision in 2 fluid ounce bottles ($T = -3.0837$, $P = 0.0024$) by 1.4 ± 5.8 g or 1.9 ± 10.3 % as compared to the directly weighed foods method (Table 16). The Bland-Altman regression plot (Figure 14D) showed a bias that was not statistically significant ($y = -0.03 (0.06) x + 0.42 (3.68)$, $R^2 = 0.0016$, $P = 0.6183$) with the difference between methods ranging from an underestimation of 15.10 g to an overestimation of 19.80 g by the RFPM. The mean difference in energy estimation between the RFPM (44.8 ± 4.7 kcal) and the directly weighed foods method (42.1 ± 4.8 kcal) was 3.0 ± 4.4 kcal (Table 16) as the energy content of the liquid food provision

is found in the dry food provision only and not the water that was mixed to prepare the liquid food provision.

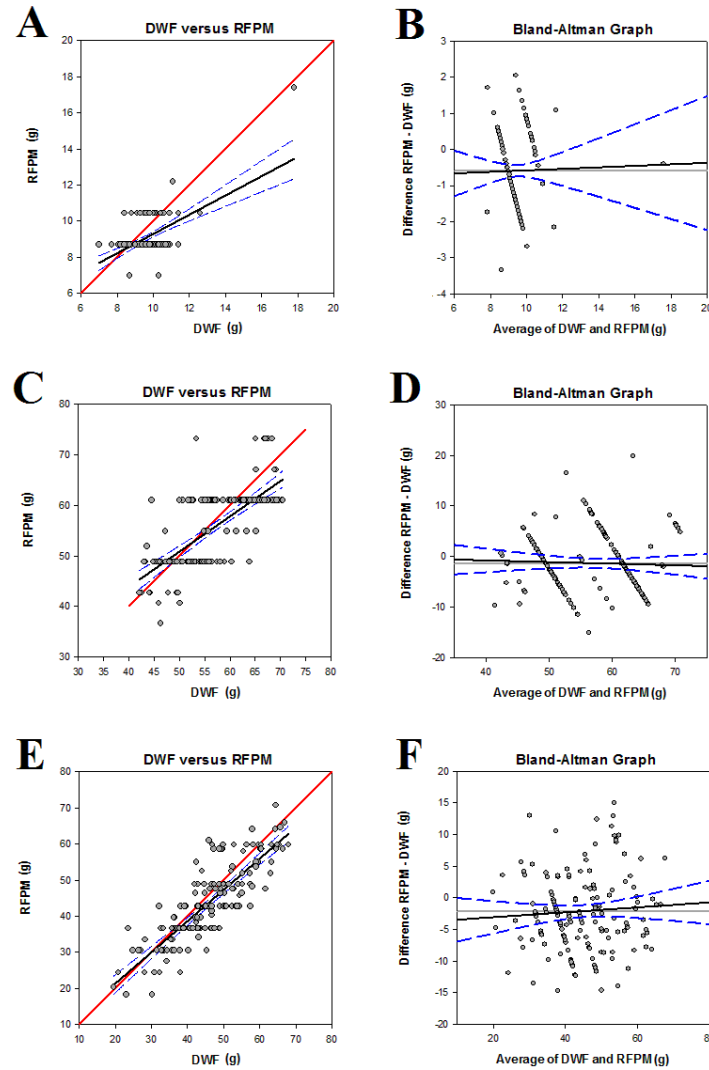


Figure 14: Bland-Altman regression analysis comparing gram weight estimated by the Remote Food Photography Method (RFPM) to the gold standard, Directly Weighed Foods (DWF) in 2 fluid ounce bottles. The figure shows: (A) Linear regression plot of Dry Food Provision (B) Bland-Altman regression plot of Dry Food Provision (C) Linear regression plot of Liquid Food Provision (D) Bland-Altman regression plot of Liquid Food Provision (E) Linear regression plot of Liquid Intake (F) Bland-Altman regression plot of Liquid Intake

The mean estimation of liquid intake in 2 fluid ounce bottles using the RFPM was 43.8 ± 10.9 g (32.7 ± 6.9 kcal) (Table 16), and the mean estimation of liquid intake in 2 fluid ounce bottles using the directly weighed foods method was 45.9 ± 10.5 g (35.7 ± 6.3 kcal) (Table 16).

As shown in the scatterplot (Figure 14E), there was a moderate association between the gram weight estimations of the RFPM and the directly weighed foods method ($R^2=0.70$, $P<0.0001$). Among 2 fluid ounce bottles in the study sample, the RFPM significantly underestimated liquid intake by 2.2 ± 6.2 g ($T=-4.4453$, $P<0.0001$) as compared to the directly weighed foods method (Table 16). This represented a mean percent difference of $4.1 \pm 14.4\%$ between methods (Table 16). The Bland-Altman regression plot (Figure 14F) that compared the means and differences in liquid intake estimations between the RFPM and the directly weighed foods method showed a bias that was not statistically significant ($y=0.04(0.05)x-3.93(2.20)$, $R^2=0.0043$, $P=0.4123$) with difference between methods ranging from an underestimation of 14.67 g to an overestimation of 15.38 g by the RFPM. The mean difference of energy estimation of liquid intake among 2 fluid ounce bottles was 2.8 ± 4.6 kcal providing a daily underestimation of liquid intake of 12 to 18 kcals by the RFPM as compared to the directly weighed foods method (Table 16).

4 fl oz

In 4 fluid ounce bottles, the mean estimation of dry food provision (two scoops of infant formula powder) was 18.1 ± 2.7 g (83.4 ± 12.5 kcals) by the RFPM and 19.3 ± 1.8 g (88.6 ± 8.1 kcals) by the directly weighed foods method (Table 16). As shown in the scatterplot (Figure 15A), there was reasonable agreement between most of the gram estimates by RFPM and the directly weighed foods method however three outliers were evident. As a result, a weak association between the two methods was observed ($R^2=0.07$, $P=0.0005$). Furthermore, the paired dependent t-test showed a significant difference in mean dry food provision estimation in 4 fluid ounce bottles ($T=-5.0716$, $P<0.0001$) between the RFPM and the directly weighed foods method with an underestimation of 1.1 ± 2.8 g or $5.5 \pm 13.9\%$ by the RFPM (Table 16). A closer inspection of the differences between the two methods, using a Bland-Altman regression plot (Figure 15B), showed that bias varied significantly with increasing gram weights ($y=0.66(0.11)x-13.44(2.10)$, $R^2=0.1802$, $P<0.0001$). The mean difference between the two methods, shown in Figure 15B, ranged from an underestimation of 7.28 g to an overestimation of 17.24 g by the RFPM. The mean difference in energy estimation of the RFPM and the directly weighed foods method was -5.2 ± 12.9 kcals providing a daily underestimation in the range of 21 to 31 kilocalories (Table 16).

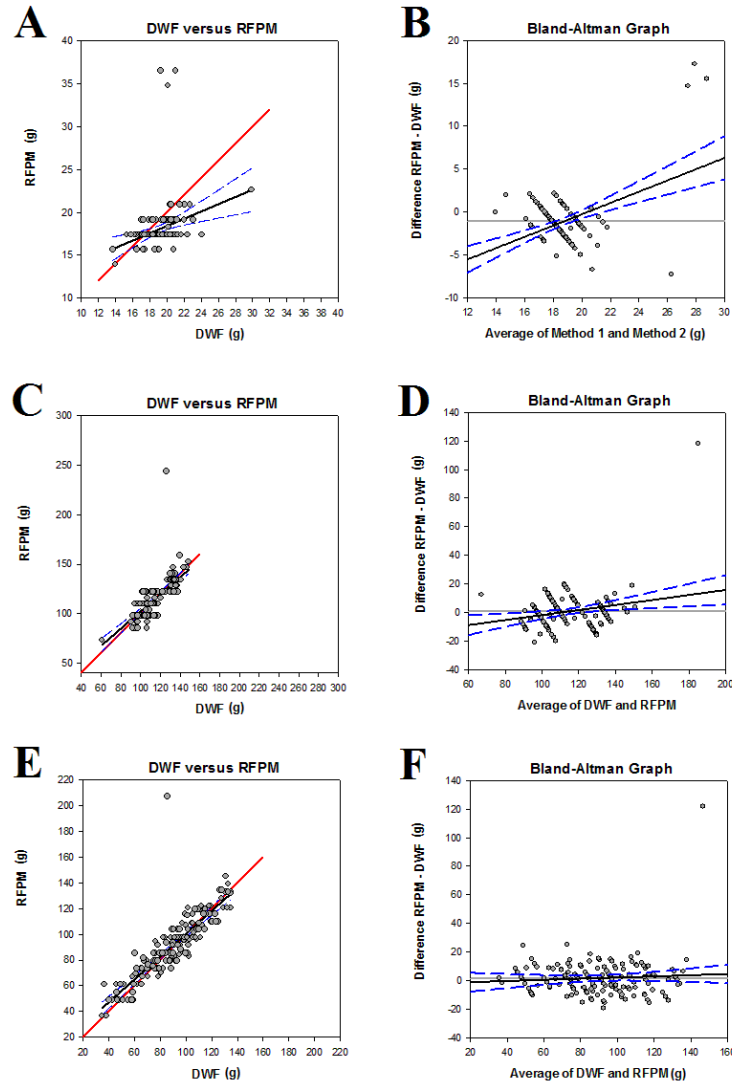


Figure 15: Bland-Altman regression analysis comparing gram weight estimated by the Remote Food Photography Method (RFPM) to the gold standard, Directly Weighed Foods (DWF) in 4 fluid ounce bottles. The figure shows: (A) Linear regression plot of Dry Food Provision (B) Bland-Altman regression plot of Dry Food Provision (C) Linear regression plot of Liquid Food Provision (D) Bland-Altman regression plot of Liquid Food Provision (E) Linear regression plot of Liquid Intake (F) Bland-Altman regression plot of Liquid Intake

Among 4 fluid ounce bottles, the RFPM and the directly weighed foods method provided mean liquid food provision estimations of 115.7 ± 19.1 g (83.4 ± 12.5 kcals) and 115.1 ± 16.3 g (88.6 ± 8.1 kcals), respectively (Table 16). As shown in Figure 15C, the RFPM and the directly weighed foods gram weight estimations were highly associated ($R^2 = 0.56$, $P < 0.0001$). The paired dependent t-test showed that there was no significant difference in estimation of liquid food provision between the RFPM and the directly weighed foods method for 4 fluid ounce bottles

with a mean error of 0.7 ± 12.8 g ($T = 0.6410$, $P = 0.5224$) (Table 16). The RFPM overestimated liquid food provision by $0.9 \pm 10.9\%$ as compared to the directly weighed foods method among 4 fluid ounce bottles (Table 16). The Bland-Altman regression plot (Figure 15D) showed a positive bias that varied significantly with increasing gram weight of dry food provision ($y = 0.18 (0.06) x - 19.61 (7.00)$, $R^2 = 0.0516$, $P = 0.0040$). The difference between the two methods, shown in Figure 15D, ranged from an underestimation of 21.10 g to an overestimation of 19.90 g by the RFPM, excluding the outlier that differed by 118.00 g. The RFPM underestimated energy content by 5.2 ± 12.9 kcals as compared to the directly weighed foods method (Table 16). As the energy content of liquid food provision is accounted for by the dry food provision and not the water used to prepare the liquid food provision, the RFPM showed a daily underestimation of liquid intake of 21 to 31 kcals by the RFPM as compared to the directly weighed foods method.

The mean estimation of liquid intake among 4 fluid ounce bottles was 91.5 ± 24.8 g (65.9 ± 16.3 kcals) using the RFPM and 90.1 ± 23.9 g (69.3 ± 11.8 kcals) using the directly weighed foods method, respectively (Table 16). As shown in the scatterplot (Figure 15E), the gram weight estimations between the RFPM and the directly weighed foods method were strongly associated ($R^2 = 0.73$, $P < 0.0001$). The paired dependent t-test showed that the RFPM did not differ significantly ($T = 1.4291$, $P = 0.1550$) as compared to the directly weighed foods method in estimating liquid formula intake among 4 fluid ounce bottles in the study sample. The RFPM overestimated liquid formula intake among 4 fluid ounce bottles by 1.5 ± 13.1 g or $2.8 \pm 16.3\%$ (Table 16). The Bland-Altman regression analysis showed a bias that was not statistically significant ($y = 0.04 (0.04) x - 2.23 (4.18)$, $R^2 = 0.0053$, $P = 0.3600$) (Figure 15F). The difference between the two methods ranged from an underestimation of 19.34 g to an overestimation of 25.20 g by the RFPM. The mean difference of energy estimation of the liquid formula intake among 4 fluid ounce bottles was 3.6 ± 10.5 g which yields an estimated error of the RFPM of 15 to 22 kcals for this serving size over the course of one day.

6 fl oz

The preparations of dry infant formula for the 6 fluid ounce bottle (three scoops of infant formula powder) were estimated with means of 26.5 ± 1.1 g (121.9 ± 5.2 kcal) by the RFPM and 28.9 ± 2.1 g (132.9 ± 9.7 kcal) by the directly weighed foods method (Table 16). As shown in the scatterplot (Figure 16A), there was a significant, yet weak correlation ($R^2 = 0.03$, $P = 0.03$) between the gram weight estimations of the RFPM and the directly weighed foods method. The RFPM significantly underestimated dry food provision ($T = -13.764$, $P < 0.0001$) by 2.4 ± 2.2 g as

compared to the gold standard method, the directly weighed foods method (Table 16). The mean percent difference of dry food provision among 6 fluid ounce bottles between the two methods was $7.9 \pm 6.9\%$. As shown in the Bland-Altman regression plot (Figure 16B), regression analysis showed that the negative bias significantly varied with the gram weights ($y = -0.10 (0.11) x + 24.41 (3.16)$, $R^2 = 0.3143$, $P < 0.0001$) such that the RFPM overestimated smaller mean gram weights and underestimated larger mean gram weights were underestimated by the RFPM, as compared to the directly weighed foods method. The mean difference between the two methods, shown in Figure 16B, ranged -13.74 g to 3.60 g. The mean difference in energy estimation between the two methods was 11.0 ± 10.1 kcals representing an underestimation of between 44 and 66 kilocalories per day.

Liquid food provision was estimated in 6 fluid ounce bottles with a mean gram weight of 180.0 ± 27.6 g (121.9 ± 5.2 kcal) using the RFPM (Table 16). The directly weighed foods method estimated liquid food provision as 174.1 ± 24.2 g (132.9 ± 9.7 kcal) (Table 16). As shown in the scatterplot (Figure 16C), the gram weight estimations between the RFPM and the directly weighed foods were highly correlated and followed the line of identity ($R^2 = 0.91$, $P < 0.0001$). Despite this strong association, the paired dependent t-test showed that the RFPM overestimated liquid food provision significantly ($T = 8.6487$, $P < 0.0001$) in 6 fluid ounce bottles by 5.9 ± 8.6 g or $3.3 \pm 4.9\%$ as compared to the directly weighed foods method (Table 16). The Bland-Altman regression plot (Figure 16D) showed positive bias that varied significantly as a function of the amount of liquid formula contained in the bottle with larger overestimations in formula bottles of larger sizes ($y = 0.14 (0.02) x - 18.33 (4.41)$, $R^2 = 0.1645$, $P < 0.0001$). The difference between the two methods ranged from an underestimation of 15.60 g to an overestimation of 32.40 g by the RFPM. Considering that dry infant formula powder accounts for energy content of the prepared formula bottles, the RFPM showed a mean energy difference of 11.0 ± 10.1 kcals between the two methods resulting in a daily error difference of 44 to 66 kilocalories.

The RFPM and the directly weighed foods method provided mean estimations of liquid intake in 6 fluid ounce bottles of 141.8 ± 42.2 g (96.0 ± 7.9 kcals) and 133.6 ± 40.7 g (102.0 ± 16.3 kcals), respectively (Table 16). The scatterplot (Figure 16E) and correlation analysis revealed a strong association between the gram weight estimations of the RFPM and the directly weighed foods method ($R^2 = 0.94$, $P < 0.0001$). The RFPM overestimated liquid intake by 8.2 ± 10.3 g and this was determined to be statistically significant by the paired dependent t-test ($T = 10.0416$, $P < 0.0001$). Mean percent difference in liquid intake between the two methods was determined as $7.0 \pm 12.4\%$. The Bland-Altman regression plot (Figure 16F), comparing liquid intake between the directly weighed foods method and the RFPM, showed a bias that was not

statistically significant ($y = 0.04 (0.02) x + 3.11 (2.84)$, $R^2 = 0.0217$, $P = 0.0638$) with the difference between the methods ranging from an underestimation of 23.70 g to an overestimation of 50.00 g by the RFPM. Energy estimation using the RFPM provided an underestimation of 5.9 ± 9.1 kcals which averages to a daily energy error range of 24 to 36 kcals.

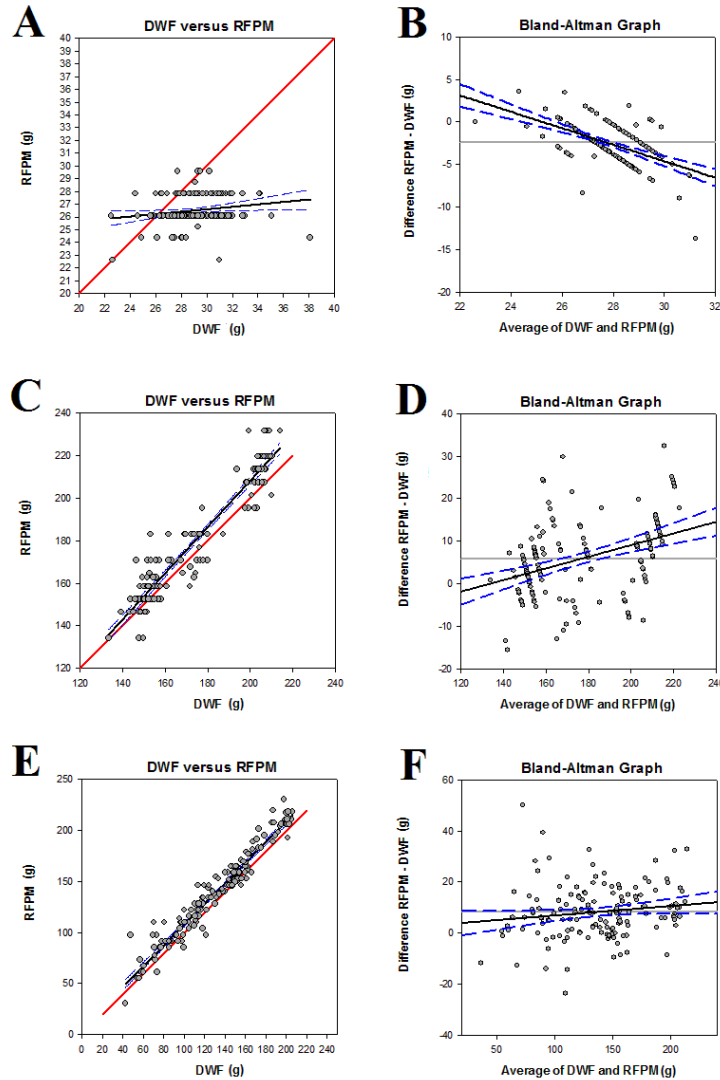


Figure 16: Bland-Altman regression analysis comparing gram weight estimated by the Remote Food Photography Method (RFPM) to the gold standard, Directly Weighed Foods (DWF) in 6 fl oz bottles. The figure shows: (A) Linear regression plot of Dry Food Provision (B) Bland-Altman regression plot of Dry Food Provision (C) Linear regression plot of Liquid Food Provision (D) Bland-Altman regression plot of Liquid Food Provision (E) Linear regression plot of Liquid Intake (F) Bland-Altman regression plot of Liquid Intake

8 fl oz

The mean estimation of four scoops of dry infant formula, or dry food provision, in 8 fluid ounce bottles was observed as 35.4 ± 1.2 g (162.7 ± 5.5 kcals) by the RFPM and 38.6 ± 3.0 g (177.6 ± 13.9 kcals) by the directly weighed foods method (Table 16). The scatterplot (Figure 17A) showed there was weak association between the gram weight estimations of the RFPM and the directly weighed foods method ($R^2=0.14$, $P<0.0001$). The mean difference between the RFPM and the directly weighed foods method was 3.3 ± 2.8 g with RFPM significantly underestimating dry food provision by $7.9 \pm 6.7\%$ ($T= -14.631$, $P<0.0001$) (Table 16). Bland-Altman regression analysis showed negative bias that varied significantly ($y= -1.16 (0.08) x + 39.50 (3.00)$, $R^2= 0.5653$, $P<0.0001$) such that the RFPM overestimated smaller gram weights and underestimated larger gram weights (Figure 17B). The mean difference between methods ranged from an underestimation of 13.76 g to an overestimation of 5.12 g by the RFPM (Figure 17B). The mean error of energy estimation of the RFPM as compared to the directly weighed foods method was 14.9 ± 12.9 kcals (Table 16). Referring to kilocalories, the mean energy estimation corresponds to an underestimation of 60 to 90 kilocalories by the RFPM as compared to the directly weighed foods method per day.

For liquid food provision for the 8 fluid ounce bottles, the RFPM provided a mean gram estimation of 244.1 ± 24.4 g (162.7 ± 5.5 kcals), and the directly weighed foods method provided a mean gram estimation of 226.6 ± 24.2 g (177.6 ± 13.9 kcals) (Table 16). As shown in the scatterplot (Figure 17C), the RFPM and the directly weighed foods gram weight estimations were highly associated ($R^2=0.82$, $P<0.0001$). A significant difference between the RFPM and the directly weighed foods method was indicated ($T= 20.9127$, $P<0.0001$) such that RFPM overestimated the directly weighed foods method by 17.5 ± 10.5 g or $7.9 \pm 4.9\%$ (Table 16). The Bland-Altman regression plot (Figure 17D) showed a bias that was not statistically significant ($y= 0.01 (0.04) x + 15.50 (8.37)$, $R^2= 0.0004$, $P= 0.8123$) with difference between methods ranging from an underestimation of 1.6 g to an overestimation of 47.5 g the RFPM. As with the dry food provision among 8 fluid ounce bottles, the mean kilocalorie difference between methods 14.9 ± 12.9 kcals resulting in an underestimation of the RFPM by 60 to 90 kilocalories per day.

The mean estimation of liquid formula intake in 8 fluid ounce bottles using the RFPM and the directly weighed foods method was 194.6 ± 45.5 g (129.7 ± 10.3 kcals) and 173.5 ± 47.4 g (136.0 ± 27.2 kcals) (Table 16), respectively. As shown in the scatterplot (Figure 17E), the gram weight estimations by the RFPM and the directly weighed foods method were highly associated ($R^2=0.93$, $P<0.0001$). The RFPM significantly differed from the directly weighed foods method ($T= 20.7925$, $P<0.0001$) in estimating liquid intake in 8 fluid ounce bottles with

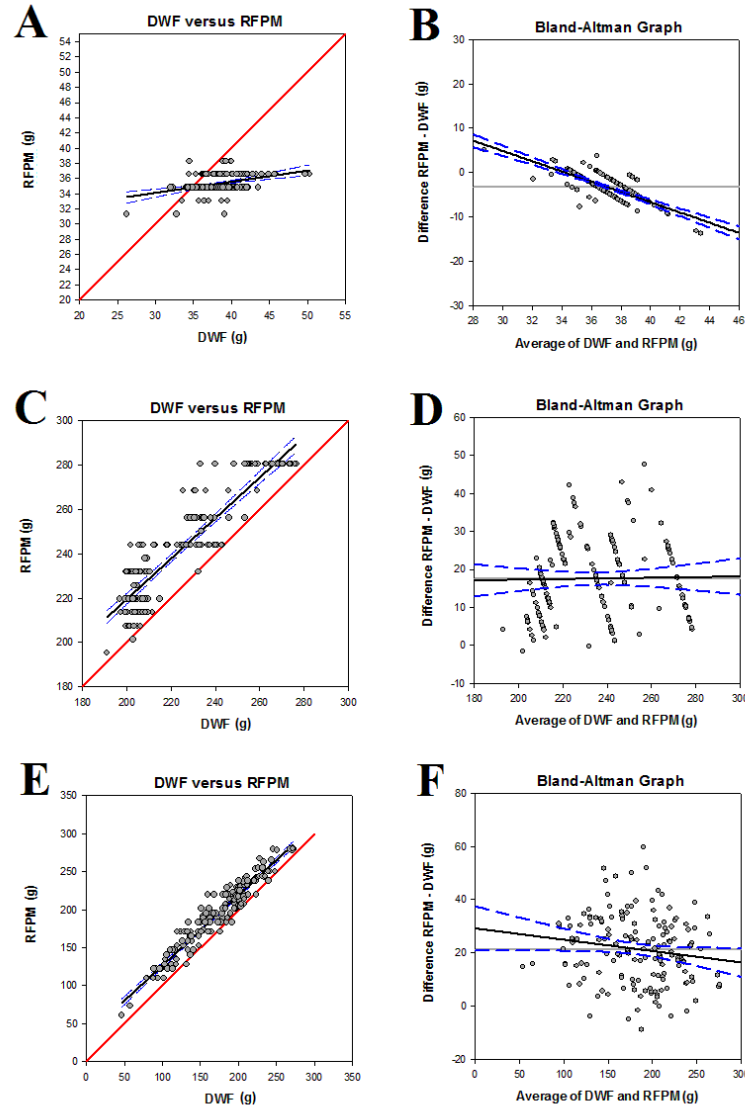


Figure 17: Bland-Altman regression analysis comparing gram weight estimated by the Remote Food Photography Method (RFPM) to the gold standard, Directly Weighed Foods (DWF) in 8 fluid ounce bottles. The figure shows: (A) Linear regression plot of Dry Food Provision (B) Bland-Altman regression plot of Dry Food Provision (C) Linear regression plot of Liquid Food Provision (D) Bland-Altman regression plot of Liquid Food Provision (E) Linear regression plot of Liquid Intake (F) Bland-Altman regression plot of Liquid Intake

mean error between the two methods of 21.2 ± 12.9 g. The RFPM overestimated by $14.0 \pm 10.3\%$ as compared to the directly weighed foods method (Table 16). The Bland-Altman regression analysis showed that bias was not statistically significant across gram weights of liquid intake ($y = -0.04 (0.02) x + 29.05 (4.18)$, $R^2 = 0.0234$, $P = 0.0542$) with difference between methods ranging from an underestimation of 8.9 g to an overestimation of 59.6 g (Figure 17F). The mean

error estimate of liquid formula intake per formula preparation was shown as 5.7 ± 12.0 kcals resulting in a daily mean error of 23 to 34 kilocalories.

Baby Bottle Study- Secondary Analysis

2 fl oz

The least squares mean estimated gram weight of a single dry scoop of formula in preparation of a 2 fluid ounce bottle prepared by caregivers was 9.90 ± 0.11 g and non-caregivers 9.58 ± 0.12 g (Figure 18). The MANOVA showed that this slight (3%) increased gram weight measured by caregivers was significantly higher than non-caregivers (adjusted $P=0.0499$) (Table 17). Importantly, the MANOVA also showed no significant difference in measured gram weight across the three trials (adjusted $P=0.0921$) and no interaction between trial number and caregiver status (adjusted $P=0.8396$) (Table 17). Adding water to the 2 fluid ounce bottle resulted in liquid food provision with least squares mean gram weights of 57.48 ± 0.87 g for caregivers and non-caregivers 57.83 ± 0.92 g for non-caregivers (Figure 19). No difference between caregivers and non-caregivers was observed in preparation of liquid food provision (adjusted $P=0.7827$). This observation was consistent across the three trials of liquid food provision (adjusted $P=0.9661$) (Table 17).

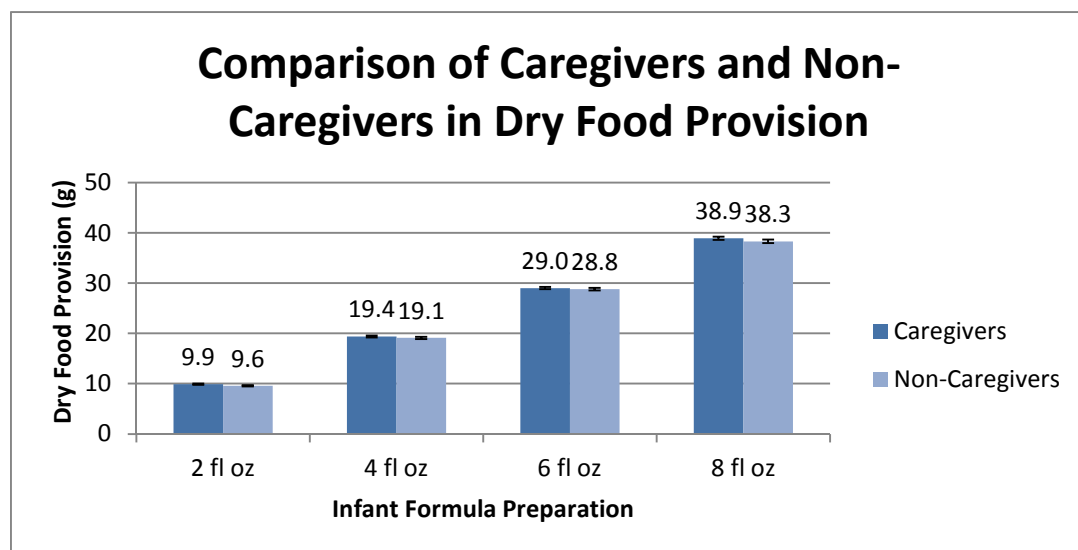


Figure 18: Least Squares Means and Standard Error of Dry Food Provision Prepared by Caregivers and Non-caregivers

4 fl oz

Caregivers and non-caregivers prepared dry food provision among 4 fluid ounce bottles with a least squares mean estimation of 19.38 ± 0.19 g and 19.12 ± 0.20 g (Figure 18), respectively. The MANOVA showed no differences in dry food provision preparation between caregivers and non-caregivers (adjusted $P=0.3409$) (Table 17). In addition, no significant difference was shown across trials of dry food provision among 4 fluid ounce bottles (adjusted $P=0.3001$). The MANOVA showed a significant interaction between trial number and caregiver status in dry food provision (adjusted $P=0.0146$) (Table 17). The least squares mean estimated gram weight of liquid food provision among 4 fluid ounce bottles by caregivers was 114.64 ± 1.80 g and non-caregivers 115.53 ± 1.90 g (Figure 19). No significant difference was observed in the preparation of liquid food provision among 4 fluid ounce bottles between caregivers and non-caregivers (adjusted $P=0.7361$) (Table 17). There was no observed effect across trials of liquid food provision among 4 fluid ounce bottles (adjusted $P=0.5952$), and the MANOVA showed no interaction between trial number and caregiver status (adjusted $P=0.6703$) (Table 17).

Table 17: MANOVA Effect Tests for the Directly Weighed Foods Method						
	Dry Food Provision			Liquid Food Provision		
	Sum of Squares	F Ratio	Adjusted P value	Sum of Squares	F Ratio	Adjusted P value
2 oz						
Caregiver	3.9900	3.9050	0.0499	4.8011	0.0764	0.7827
Trial #	4.9509	2.4227	0.0921	27.3164	0.2172	0.8050
Trial #*Caregiver	0.3577	0.1751	0.8396	4.3398	0.0345	0.9661
4 oz						
Caregiver	2.7199	0.9127	0.3409	31.0234	0.1140	0.7361
Trial #	7.2304	1.2131	0.3001	283.3440	0.5207	0.5952
Trial #*Caregiver	25.9001	4.3456	0.0146	218.2597	0.4011	0.6703
6 oz						
Caregiver	0.6492	0.1472	0.7017	109.7549	0.1824	0.6699
Trial #	12.3104	1.3960	0.2507	58.0804	0.0483	0.9529
Trial #*Caregiver	9.8488	1.1169	0.3300	13.4524	0.0112	0.9889
8 oz						
Caregiver	13.4387	1.4729	0.2268	285.6789	0.4737	0.4923
Trial #	9.2813	0.5086	0.6023	162.9344	0.1351	0.8737
Trial #*Caregiver	21.5937	1.1834	0.3090	141.2892	0.1171	0.8895
Sum of Squares, F Ratio, and Adjusted P value were calculated using Multivariate Analysis of Variance on JMP 10.0.0 (SAS Institute, Cary, North Carolina, United States). P values in bold text were statistically significant (alpha was set at 0.05).						

6 fl oz

The least squares mean estimation of dry food provision among 6 fluid ounce bottles prepared by caregivers was 28.97 ± 0.23 g and non-caregivers 28.84 ± 0.24 g (Figure 18). The MANOVA showed no difference in preparation of dry food provision between caregivers and non-caregivers (adjusted $P=0.7017$) (Table 17). The MANOVA also showed no significant difference in gram weight estimations across the three trials (adjusted $P=0.2507$) (Table 17). After preparing liquid food provision by adding water to the dry food provision, there were no differences between caregivers and non-caregivers (adjusted $P=0.6699$) (Table 17) with least squares mean estimations of 173.33 ± 2.68 g by caregivers and 174.99 ± 2.83 g by non-caregivers (Figure 19). No significant differences were observed across the trials among 6 fluid ounce bottles (adjusted $P=0.9529$) (Table 17). The MANOVA showed no interaction between trial number and caregiver status for dry food provision (adjusted $P=0.3300$) and liquid food provision (adjusted $P=0.9889$) (Table 17).

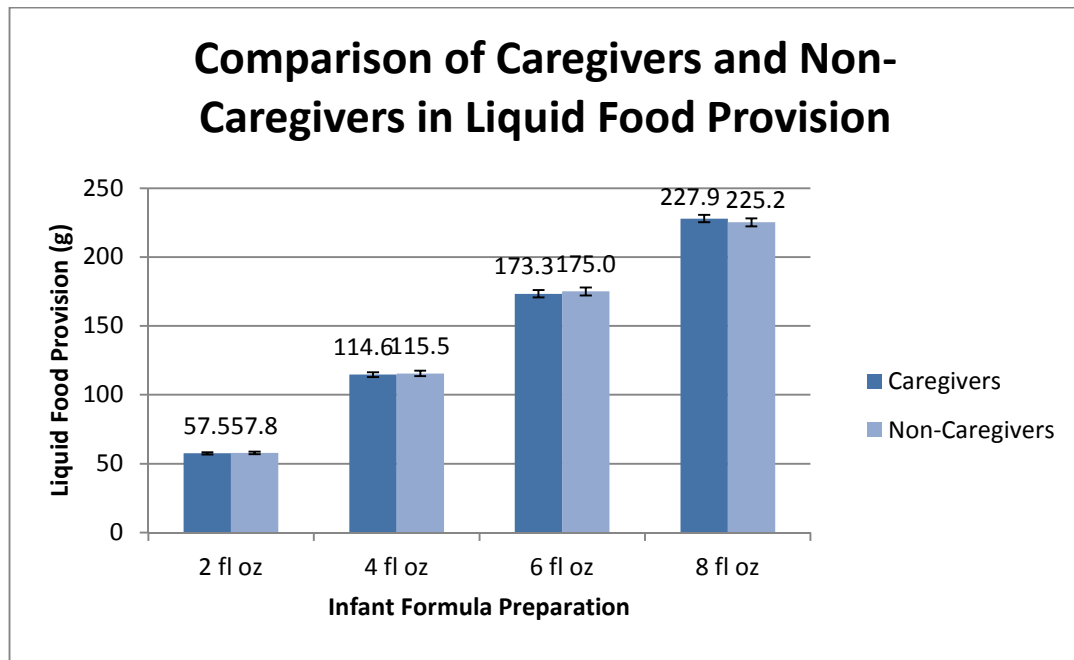


Figure 19: Least Square Means and Standard Error of Liquid Food Provision Prepared by Caregivers and Non-caregivers

8 fl oz

Caregivers and non-caregivers prepared dry food provision among 8 fluid ounce bottles with a mean estimation of 38.90 ± 0.33 g and 38.32 ± 0.35 g, respectively (Figure 18). The MANOVA showed no differences in dry food provision preparation between caregivers and non-caregivers (adjusted $P=0.2268$) and across trials of dry food provision among 8 fluid ounce bottles (adjusted $P=0.6023$) (Table 17). The MANOVA showed no interaction between trial number and caregiver status in dry food provision (adjusted $P=0.3090$) (Table 17). After adding water to dry food provision, no difference was observed in prepared liquid food provision between caregivers and non-caregivers (adjusted $P=0.4923$) (Table 17) with least squares mean estimations of 227.86 ± 2.68 g by caregivers and 225.17 ± 2.84 g by non-caregivers (Figure 19). The MANOVA showed no effect across trials of liquid food provision among 8 fluid ounce bottles (adjusted $P=0.8737$), and no interaction between trial number and caregiver status of liquid food provision (adjusted $P=0.8895$) (Table 17).

CHAPTER 5: DISCUSSION

Measuring infant food intake in near real-time with objective methods is important not only for the assessment of growth and development¹³, but also in the prevention of infant overfeeding which may be instrumental in preventing childhood obesity^{10,12}. In the Baby Bottle study, a relatively new validated method for assessment of energy intake, the Remote Food Photography Method (RFPM), was tested to establish if the RFPM is an appropriate tool for measuring infant food intake. Therefore, the overarching objective was to assess if the RFPM can accurately estimate simulated infant formula intake compared to the gold standard, the directly weighed foods method. Secondary objectives of the study were to evaluate the inter- and intra-individual variability in infant formula preparation and to investigate the variability in infant formula preparation between caregivers and non-caregivers.

The RFPM significantly underestimated dry food provision among all dry food provision preparations, but the mean difference in estimations by the RFPM as compared to the directly weighed foods method remained small ranging from $-5.7 \pm 10.2\%$ among 2 fluid ounce dry food provision preparations to $-7.9 \pm 6.7\%$ among 8 fluid ounce dry food provision preparations. The mean difference of the RFPM with respect to the directly weighed foods method among liquid food provision preparations exhibited a trend of increasing error ranging from $-1.9 \pm 10.3\%$ among 2 fluid ounce liquid food provision preparations to $7.9 \pm 4.9\%$ among 8 fluid ounce liquid food provision preparations. The mean error estimation of the RFPM with respect to the directly weighed foods method among liquid intake exhibited a similar trend to the liquid food provision preparations with increasing error ranging from $-4.1 \pm 14.4\%$ among liquid intake in 2 fluid ounce bottles to $14.0 \pm 10.3\%$ among liquid intake in 8 fluid ounce bottles. The RFPM provided accurate estimations, within 10% error of the directly weighed foods method, of liquid intake among 2 fluid ounce bottles, 4 fluid ounce bottles, and 6 fluid ounce bottles. Mean estimation of liquid intake among 8 fluid ounce bottles was not within 10% error of the directly weighed foods method. There was increasing error of the RFPM as compared to the directly weighed foods method in liquid intake with increasing size of the formula bottles. In addition, findings from the Baby Bottle study showed that the RFPM failed to be flexible with precision in estimations of dry food provision, liquid food provision, and liquid waste. In some cases, there were identical gram weight estimations for various bottles of similar sizes using the RFPM although their measurements from the directly weighed foods method differed. Although this was a systematic error within the RFPM, study findings indicate that the gram weight estimations by the RFPM were correlated with the measurements by the directly weighed foods method. Although majority

of analyses between the RFPM and the directly weighed foods method were significantly different in the present study, agreement between methods was strong with the RFPM overestimating combined bottles of liquid intake by only $4.9 \pm 15.0\%$ as compared to the directly weighed foods method.

Gram estimations of the two methods were translated into energy estimations, and error estimations of energy intake were calculated from the energy estimations. This was accomplished by accounting for the energy content of liquid intake and multiplying that by 4.5977 g/kcal to provide equivalent kilocalories. The RFPM underestimated energy intake by 4.6 ± 9.5 kcals as compared to the directly weighed foods method. Since dry infant formula powder is the source of energy in infant formula, underestimation in dry food provision is likely the source of kilocalorie error in infant intake estimations by the RFPM. The mean error of energy intake provides a daily underestimation of 20 to 30 kcals by the RFPM, illustrating that there is minimal clinical difference between the two methods on a daily scale.

Currently available, objective methods for estimating energy intake in infants are fairly accurate but have reasonably high burden and cost^{8,9}. Typically, there is small overestimation with objective methods in infants due to losses from spit up, spillage during feeding, or drool⁷². The directly weighed foods method, one of the most accurate methods for measuring infant food intake, has been shown to be within 5% of estimated energy requirements^{9,14,16}. Test weighing has been shown to be the most appropriate method for assessing food intake in breastfed infants^{17,71,72}. In infants, test weighing has been shown to overestimate the directly weighed foods method by 8% using a mechanical scale and by 1% using an electronic scale^{18,72}. Doubly labeled water has been shown to overestimate energy intake by 8% in formula fed infants⁷⁶, by 6% in children under two and a half years of age¹⁹, and by 7% in infants aged six to twelve months²⁰ as compared to the directly weighed foods method. In infants, doubly labeled water can be used to measure total energy expenditure, energy intake and energy content⁷³, but with high cost and low accessibility to technology and instrumentation necessary for this method, it is not practical for widespread clinical use^{7,8}.

Subjective methods are useful in clinical settings for ease and wide distribution to large populations, but these methods rely on memory and portion estimation making them less accurate than objective methods⁷. Estimated food diaries have been shown to underestimate the directly weighed foods method by 4% in infants²⁰. With this self-report method, there is potential for poor portion estimation, forgetting foods⁷, and under eating²¹. In infants, twenty-four hour diet recalls have been shown to overestimate energy intake by 13% as compared to the directly weighed foods method¹⁴. Major disadvantages of the twenty-four hour diet recall method include relying

on the patient's memory and ability to estimate portion sizes and that these recalls only report feeding in the previous twenty-four hours¹⁴. Food frequency questionnaires are commonly used on a population-based level^{16,74}, and they have been shown to overestimate energy intake by 25% in infants aged twelve months as compared to the directly weighed foods method¹⁶.

In comparison to these existing methods, the Baby Bottle study showed that the RFPM is an acceptable method for assessing infant formula intake with mean error within 10% of the directly weighed foods method among 2 fluid ounce bottles, 4 fluid ounce bottles, and 6 fluid ounce bottles. As previously mentioned, the mean error was not within 10% among 8 fluid ounce bottles, but a mean error of 14% is an improvement over current subjective methods. With evidence from the Baby Bottle study, the RFPM has a higher degree of accuracy in assessing infant formula intake as compared to the subjective methods and similar accuracy as compared to the objective methods. It was determined that the RFPM provides accurate estimations of energy intake in infants and is comparable to the directly weighed foods method^{14,42}, test weighing^{17,18}, and the doubly labeled water method^{19,72,76} however the RFPM has decreased cost, burden, and time commitment from individuals as compared to these methods^{8,9}. In addition, the RFPM allows for photo capturing before and after feeding with continued communication with analyzers and near real-time analysis to increase compliance and accuracy as compared to self-report methods that rely on memory^{14,16}. One major strength of the RFPM as compared to subjective methods is that it does not rely on portion estimation by the individual user or caregiver which is necessary for accurate completion of a food diary, dietary recall, or food frequency questionnaires, but portion estimation can more accurately be estimated by trained analyzers⁷. With the RFPM, diet quality and quantity can be assessed over a short or long period, and kilocalorie, macronutrient, and micronutrient intakes can be expressed^{7,22}. As compared to test weighing^{17,71,72}, a significant limitation with using the RFPM to assess energy intake in breastfed infants is the inability to capture and analyze photographs of breastfeeding sessions and the inability to determine energy content of breast milk provided to infants. As with other objective methods, estimation error includes insensible losses due to inconsistency with clothing changes, evaporation from the skin, losses from spit up, and infant movement^{17,76}.

Since parents and caregivers are responsible for influencing infant feeding behavior^{4,8,10} and contributing to healthy growth and development⁴, the variability of infant formula preparation between caregivers and non-caregivers was assessed in the Baby Bottle study. Fifty-three individuals, 28 caregivers and 25 non-caregivers, completed the study. There were no significant demographic differences between the caregivers and non-caregivers, and, importantly, the study sample included a wide age range (18 to 71 years of age) and wide BMI range (17 to 56

kg/m²). Caregivers and non-caregivers did not differ significantly in the preparation of dry food provision or liquid food provision among all bottle sizes except for the preparation of dry food provision among 2 fluid ounce bottles. Additionally, there were no significant differences in dry food provision or liquid food provision preparations within individuals across the three trials indicating that there was low individual variability across all preparations of dry food provision and liquid food provision. Accuracy of the RFPM in infants relies on parents and caregivers to use the RFPM before and after infant feeding to provide food provision and waste photographs and descriptions of consumed foods. Evidenced in the present study, the RFPM is a suitable method when parents and caregivers follow RFPM training and photo capturing procedures.

The Baby Bottle study included a few limitations. As discussed in *Chapter 1*, whole milk powder as compared to powdered infant formula was used in the Baby Bottle study. Since study design required formula contents to be discarded, whole milk powder proved to be a cost-effective alternative. In future studies, the analysis of actual infant formula should confirm that there was no difference in estimation by the RFPM between whole milk powder and powdered infant formula. In addition, the study design required the order of bottle preparation to be standardized. An essential step of the Baby Bottle study to assess the accuracy of the RFPM was to capture and analyze all steps of infant formula preparation including the dry food provision as dry infant formula powder accounts for the energy content of prepared infant formula. Since there was low variability between preparations of dry food provision across trials, it may be appropriate to eliminate the need for a dry food provision photograph by using established standard estimations of dry food provision to calculate energy estimations within liquid intake. With the analysis of gram weights of a single scoop of dry powdered formula and the comparison of these gram weights to the standard size of a single scoop of 8.7 g as indicated in the Similac Advance nutrition information, it was observed that there is a systematic error by humans in the preparation of a single scoop of dry powdered formula. Individuals do not accurately measure 8.7 g in a single scoop of dry powdered formula for every preparation of a scoop, but preparation of a single scoop ranged from about 7 g to 11 g. Of notable importance, this error represents the error in any given scoop within a formula bottle which will be compounded with the addition of several scoops of powdered formula required for the preparation of larger volumes of infant formula. Additionally, since photographs of a formula bottle containing two scoops of dry powdered formula and a formula bottle containing 4 fluid ounce of prepared formula were used as the standard photographs in the Food Photography Application©, the RFPM estimated liquid intake accurately in comparison to the directly weighed foods method among 4 fluid ounce bottles. Error estimations by the RFPM were higher among 2 fluid ounce bottles, 6 fluid ounce bottles, and 8

fluid ounce bottles, which may be explained by using the two scoop and 4 fluid ounce standard photographs as compared to standard photographs of the respective bottle sizes for portion estimation. One future improvement to the RFPM infant protocol developed in the Baby Bottle study is to use standard photographs of the appropriate bottle sizes rather than using only the arbitrarily chosen standard photographs. For example, this would allow for the ratio of a test photograph of a 6 fluid ounce bottle to be estimated against the standard photograph of a 6 fluid ounce bottle indicating that the error may be reduced compared to the error observed in liquid intake among 6 fluid ounce bottles in the present study. Utilization of the appropriate formula bottle size standard photographs, as compared to the arbitrarily chosen standard photographs from the Baby Bottle study, may allow for the RFPM to be as accurate as possible, limiting the analysis error in the Food Photography Application©. With respect to the recommended RFPM infant procedures in the present study, there were difficulties in the analysis and estimation of dry powder in some instances when the bottle was not shaken to allow the dry powdered formula to be more uniformly distributed in the bottle. In addition, some formula bottle photographs were taken at a sizeable distance from the bottle, impeding accurate estimation of the contents of the bottle. Future RFPM infant instructions should recommend caregivers to evenly distribute the powder in the formula bottle and to maintain a suitable distance from the bottle for more accurate estimation by the analyzer. Another limitation of the present study was that it was restricted to investigating the ability of the RFPM in assessing infant formula intake rather than all foods that infants may consume after six months of age. Assessing infant formula intake was an obvious initial step for application of the RFPM in infants. Moving forward, the RFPM should be tested for its ability to assess infant feeding at all stages of development because early feeding practices from birth to two years of age have been shown to contribute to long term feeding behavior¹⁰, and the ability to assess the pattern of early feeding may be instrumental in monitoring growth and development throughout childhood.

The Baby Bottle study was a validation study to assess infant formula intake, and therefore, a protocol for the use of the RFPM to assess infant formula was developed. The RFPM infant formula protocol included a few novel features specifically for infants to improve the accuracy of the method. As part of the basic RFPM infant formula procedure, dry food provision, liquid food provision, and liquid waste photographs were captured with emphasis on accurately capturing the dry food provision photograph as it accounts for the energy content of prepared infant formula bottles. Additionally, the RFPM infant formula standard card was developed to increase accuracy by the RFPM in assessing the number of scoops contained in food provision by allowing individuals to report the number of scoops of powder dry formula added to the bottle.

Moving forward, accurate estimation of infant formula intake can be achieved by capturing photographs of liquid food provision and liquid waste and using the RFPM infant formula standard card in the liquid food provision photograph to report the appropriate number of dry powder scoops. Successful use of the RFPM infant formula standard card allows for adequate estimation of the dry food provision so that energy content can be accurately estimated, even with the absence of a dry food provision photograph. As previously mentioned, another possible improvement to the current RFPM infant formula protocol is to use standard photographs for analysis that correspond to formula bottles in test photographs. Additionally, future RFPM infant formula instructions will include specifications of evenly distributing infant formula within the formula bottle and capturing photographs at an appropriate distance. Caregivers will be instructed to report the number of dry formula powder scoops measured for the infant formula preparation and to mix the liquid food provision formula bottle to achieve uniform consistency throughout the formula bottle.

In conclusion, the RFPM was shown to be a viable method in assessing infant formula intake with increased accuracy as compared to self-report methods and decreased cost and burden as compared to objective methods. While validation studies in free-living infants and caregivers represent the obvious next step, the Baby Bottle study provided important information to provide a potential future protocol for RFPM to measure infant food intake for research or clinical purposes. Future studies are needed to investigate the RFPM infant formula protocol with the proposed changes and improvements. After validation of the RFPM in free-living infants, the RFPM can be used in research and clinical settings as a tool for monitoring infant food intake.

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APPENDIX A: PROTOCOL

PBRC IRB# 12035

-Protocol-November 14, 2012-V.02-

Remote Food Photography Method in Infants: A Pilot Study "Baby Bottle"

PROTOCOL

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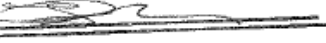
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1. SUMMARY

The objective of this study is to validate the Remote Food Photography Method (RFPM) to assess food intake in formula fed infants. Up to 75 men and women will participate in this 2-week study. Participants will be asked to prepare, in random order, multiple servings of 2 fluid ounce, 4 fluid ounce, 6 fluid ounce and 8 fluid ounce bottles of infant formula on 2 occasions about 1 week apart.

In this observational study, we will test the primary aim to assess if the RFPM can accurately estimate simulated food intake compared to the gold standard— directly weighed foods. A secondary aim is to evaluate the inter- and intra- individual variability in infant formula preparation.

2. BACKGROUND AND SIGNIFICANCE

2.1. Infant Food Intake

Measuring food intake for infants is challenging because foods and eating patterns are constantly changing during the first two years of life. In addition, food intake during the first two years of life dramatically differs from food intake during the remainder of life^[1], where most of the methods for assessing food intake are focused. Infants begin feeding with exclusively human milk or infant formula. Pureed foods and, then, solid foods are introduced gradually within the first year of life. Challenges and inconsistencies with measuring food intake in infants include losses from spit up and losses from infant movement^[2]. Test weighing is commonly used to measure food intake in early infancy as this method is believed to be the most accurate behind doubly labeled water^[3]. Test weighing involves weighing infants before and after an observed feeding with the difference in weight indicative of food intake. Test weighing may be used in infants that are breastfed and formula fed, but studies suggest it is most frequently used during early infancy^[4]. Twenty-four hour dietary recalls and the directly weighed food method are also commonly used to determine energy intake in infants and toddlers. A previous study in infants and toddlers showed that the directly weighed food method estimated energy intakes within 5% of estimated energy requirements and that the 24 hour dietary recalls overestimated energy intakes in infants by 13% and in toddlers by 29%^[5]. Research suggests that established methods for evaluating infant food intake are useful, but their limitations and challenges show a need for new methods to be developed and validated.

According to the Federal Food, Drug, and Cosmetic Act (FFDCA), infant formula should be used solely as food for infants as a complete or partial substitute for human milk. The majority of formula available in the United States is sold in powdered form. Caregivers mix powdered formula with water to prepare formula for infants to consume. While the FDA regulates the nutrient content of infant formula, there may be variability in formula intake with differences in formula preparation by caregivers and feeding patterns^[6].

2.2. Remote Food Photography Method

The Remote Food Photography Method (RFPM) is used to measure energy and nutrient intake utilizing digital photography of food selection and plate waste to estimate food intake. When using the RFPM, individuals use Smartphones to capture images of their food selection and plate waste. The images are then sent to study staff using a wireless network. Food images are stored in a computer program called the Food Photography Application^[6]. The food images acquired from digital photography are compared to known portion sizes of those food images to estimate energy and nutrient intakes^[7]. The use of digital photography and the RFPM has been validated in adults^[6] and can be readily adapted to other populations including infants and toddlers.

2.3. Summary

Food intake is crucial during the first two years of life because it is a time of constant growth and development. Measuring food intake in infants is challenging due to constant changes in eating patterns and large variability in

food selection. Establishing accurate methods to estimate food intake in infants is important for establishing effective feeding practices, supporting adequate growth and development and to help understand the role of food intake in the development of childhood obesity. Digital photography and the RFPM is used to measure energy and nutrient intake in adults, but these methods have not been used to measure energy and nutrient intake in infants from birth to 12 months. The primary objective of this study is to determine the reliability and validity of the RFPM to assess food intake in formula fed infants.

2.4. Study Aims

Primary Aim: To assess if the RFPM can accurately estimate simulated food intake compared to the gold standard— directly weighed foods.

Secondary Aim: To evaluate the inter- and intra-individual variability in infant formula preparation.

Exploratory Aim: To investigate the variability in infant formula preparation between caregivers and non-caregivers of infants. A caregiver will be defined as an individual who is a parent, grandparent, sibling, aunt or uncle, or nanny or babysitter who has provided care to an infant within the last 12 months.

3. RESEARCH DESIGN

This is an observational study with the overall objective to determine the reliability and validity of the RFPM to assess food intake in formula fed infants.

4. STUDY POPULATION

4.1. Participants

Up to 75 men and women will be recruited to participate in a 2-week pilot study involving infant formula preparation. Participation is open to all adult individuals.

4.2. Eligibility Criteria

Participants are eligible to participate in this study if they are:

- ≥ 18 years of age
- Willing to complete 2 study visits at Pennington Biomedical (PBRC)

5. RECRUITMENT

Subjects will be recruited from within PBRC and the Greater Baton Rouge Area using the PBRC website advertisement and an email blast to Pennington Biomedical employees and Recruiting list serv.

6. ASSESSMENT SCHEDULE AND PROCEDURES

Potential participants will complete a telephone or email screen to determine basic eligibility prior to enrollment. After signing the study consent and HIPAA authorization, participants will be required to complete 2 study visits separated by about 1 week (5-10 days). Study visits can be performed at any time of the day although the time of day will be standardized for each participant. Participants will complete several procedures during the study visits. See Table 1, Schedule of Procedures.

Table 1: Schedule of Procedures	Screening	Testing	
	Telephone Screen	Visit 1	Visit 2
Eligibility Evaluation	X		
Informed Consent and HIPAA		X	
Anthropometry (Height/Weight)		X	
RFPM Training		X	
Infant Formula Preparation		X	X
Remote Food Photography Method		X	X
Directly Weighed Food Method		X	X

6.1. Screening

Potential participants can use a webscreener to screen online for the Baby Bottle study; available at www.pbrc.edu/clinicaltrials. Webscreener questions are designated by the study investigators and include basic eligibility criteria (e.g. age, BMI, sex, race) and general questions designated by the Research Computing Group. Interested and eligible subjects will be assigned a subject identification number (PBRC ID number) and scheduled for Visit 1. Where possible, the study informed consent form will be emailed to eligible subjects with intent for the participant to read and review the informed consent form prior to Visit 1.

6.2. Visit 1

Volunteers will report to PBRC Outpatient Clinic and Ingestive Behavior Laboratory (IBL) to complete Visit 1. Upon arrival, potential participants will read and review the informed consent form. After all questions and concerns are addressed and prior to study procedures being conducted, interested participants will provide written informed consent and will sign the HIPAA form. Body weight and height will be measured and body mass index will be calculated according to the standard procedures of PBRC. Participants will be asked to complete a questionnaire to assess parent/caregiver status. The questionnaire can be found in Appendix 1. The questionnaire is expected to take up to 5 minutes to complete. After completing the self-reported questionnaire, participants will be trained to use RFPM to capture photos.

Each participant will, in random order, prepare 2 bottles of infant formula measuring 2 fluid ounces, 4 fluid ounces, 6 fluid ounces, and 8 fluid ounces. A total of 8 formula bottles will be prepared at Visit 1. Manufacturers' instructions state that 1 level scoop of powdered formula (8.8g) yields a 2 fluid ounce bottle, 2 unpacked level scoops of powdered formula (17.6g) yields a 4 fluid ounce bottle, 3 unpacked level scoops of powdered formula (26.4g) yields a 6 fluid ounce bottle, and 4 unpacked level scoops of powdered formula (35.2g) yields an 8 fluid ounce bottle. The Remote Food Photography Method and Directly Weighed Food Method will be utilized for each formula preparation as described below. Food weights (to the nearest tenth of a gram) will be measured using METTLER TOLEDO PB3001 scales. For each bottle, the participant will use the formula scoop provided to measure the required amount of powdered formula. The participant will dispense the correct number of scoops of powdered formula into the formula bottle depending on which size bottle is being prepared. After all powdered formula is dispensed into the clear formula bottle, the participant will capture a photo of the formula bottle with powdered formula only. This will be referred to as food provision. PBRC staff will weigh the formula bottle with powdered formula only. The participant will pour water into the bottle and mix the formula. Then, the participant will capture a photo of the prepared formula bottle. This will also be called food provision. PBRC staff will weigh the prepared formula bottle. Then, the PBRC staff will discard a random amount of the prepared formula. The participant will capture a photo of the formula bottle after the random amount of the prepared formula has been discarded. This will be referred to as waste. Then, PBRC staff will weigh the formula bottle. The captured photos will be used to determine if the RFPM accurately estimates simulated infant food intake.

which will be evaluated as food provision minus waste. This procedure will be repeated to assess intra-individual variability.

6.3. Visit 2

Participants will be asked to return to the IBL approximately 5 to 10 days later to complete Visit 2. At Visit 2, participants will be instructed to repeat the infant formula preparations from Visit 1. Participants will prepare 4 formula bottles at Visit 2. Each participant will, in a random order, prepare bottles that will provide 2 fluid ounces, 4 fluid ounces, 6 fluid ounces, and 8 fluid ounces of infant formula bottles.

7. MEASURES AND OUTCOME ASSESSMENTS

7.1. Anthropometrics

Height and body weight will be measured at Visit 1 using standard procedures of PBRC. Non-fasting body weight will be recorded. Body mass index will then be calculated from the recorded height and body weight.

7.2. Self-Report Questionnaires

A questionnaire that will assess parent/caregiver status will be administered to the participants at Visit 1 (Appendix 1). The questionnaire will assess demographic information and parent/caregiver status. A caregiver will be defined as an individual who is a parent, grandparent, sibling, aunt or uncle, or nanny or babysitter who has provided care to an infant within the last 12 months. A log will be kept throughout the study to document the distribution of caregiver status among participants. Effort will be made to maintain balance between the number of caregivers and the number of non-caregivers participating in the study.

7.3. Remote Food Photography Method

The RFPM procedures are detailed in the Background and Significance Section. In brief, participants will use RFPM to capture images of the powdered formula in the bottle, the prepared formula bottle, and the formula bottle after discarding waste.

7.4. Directly Weighed Food Method

The directly weighed food method is a useful method for estimating food intake and simulated waste in research and clinical settings. The formula bottle with powdered formula, the prepared formula bottle, and the formula bottle after discarding waste will be directly weighed to the tenth of a gram using METTLER TOLEDO PB3001 scales in the IBL.

8. PARTICIPANT SAFETY AND CONFIDENTIALITY

8.1. Risks To Participant

This study does not involve major risk to participants. Efforts to minimize the potential risks of the assessment methods and outcome variables include frequent monitoring by the investigators. The study procedures include:

- Body weight. There is no risk to participants who record their body weight.
- Height. There is no risk to participants who record their height.
- Self-report Questionnaires. There are no anticipated risks from completing self-report questionnaires. It is estimated that the questionnaires will take 5 minutes to complete.
- Formula Preparation. There is no risk to participants who prepare formula.

8.2. Adverse events

Serious adverse events in this study are defined to include: death, a life-threatening adverse experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant

disability/incapacity. In this study, an adverse event or experience is defined as any health-related unfavorable or unintended medical occurrence that happens after screening. AE data will be analyzed quarterly, but serious or life-threatening adverse events require immediate reporting and follow-up. We anticipate most adverse events will be mild and the participant will be able to resume activities within a day or two of reporting the event. Adverse Event reporting will follow the requirements of the IRB of the Pennington Biomedical Research Center. Serious adverse events will be reported within 48 hours. Other adverse events that are not serious but are unexpected and are associated with the study procedures will be reported within 10 days.

8.3. Confidentiality

All volunteers are assured of their anonymity and confidentiality both verbally and in the informed consent form. The clinical facilities are strictly limited to the staff of the research institution and to research volunteers. This is accomplished by a variety of stringent security measures. All medical records are stored in locked areas. Access to these areas is limited to the clinical support staff, director of the clinical facilities, and the PIs. Volunteers' medical records are filed according to ID numbers. All forms on the chart, with the exception of consent form, display only the ID number. Electronic data storage is similarly restricted with only the PIs and authorized persons having access to databases containing confidential clinical records, i.e. those containing name, social security number, or other identifying information.

Data, including body weight and some demographic information will be collected from participants. Data are confidentially collected from study participants and are only used for research purposes. All records are kept in locked file cabinets, and participant data can be identified only by number. Data are used only in aggregate, and no identifying characteristics of individuals are published or presented.

9. DATA ANALYSIS PLAN

9.1. Power Analysis

We will test the primary aim to assess if the RFPM can accurately estimate simulated food intake compared to the gold standard— directly weighed foods. Power calculations were performed on the main outcome variable, which is the simulated food intake. Two measures of food provision will be obtained: powdered formula and prepared formula, the latter of which is created by adding water to the powdered formula. Food waste is defined as the liquid formula remaining in a bottle after simulating feeding (random discard from Gaussian distribution of 25-100% of prepared bottle). Food intake is food provision minus food waste. A secondary aim is to evaluate the inter- and intra- individual variability in infant formula preparation so this study is also appropriately powered to detect differences in the grams of powdered dry formula in the bottle.

A power analysis was conducted for the Bland and Altman procedure^[9] that will be used to determine if the RFPM significantly over or underestimates food provision, waste, or intake and if the error associated with the RFPM varies over the amount of food provision, waste, and intake. Determining if error variance differs over levels of intake is critical to examining validity and accuracy; hence, the sample size was established based on the regression analysis used to do so since it required the largest number of participants.

A power analysis was conducted for the Bland-Altman regression analysis^[8]. The power analysis indicated that an R^2 of .14 can be detected with 53 participants (power = .80) and this is considered acceptable based on studies that used Bland-Altman analysis on biological parameters^[9]. This research indicates that poor measures frequently have $R^2 \geq 0.16$; therefore, sample size of 53 would yield satisfactory statistical power in the analyses for the primary aim. The primary aim was used for power calculations since sample size was based on the least powered endpoint (i.e. aim 1).

Paired dependent t-tests will determine if the RFPM has significant error; i.e., if RFPM estimates differ significantly from weighed food. Variance estimates for the power analyses were obtained from our laboratory [10] and assumptions included alpha equal to 0.05, two-tailed tests, and anticipated sample size with 5% attrition (56 subjects will be begin the study anticipating we will have a final sample size of 53 subjects.) Again, power of .80 was considered acceptable.

Power for intake (provision minus waste) of prepared formula was calculated with variance estimates for intake of beverages from our laboratory^[10]. As illustrated in Table 2, with 53 participants and the assumptions listed above, we have 80% power to detect a 4.95 g difference between RFPM estimated and directly weighed prepared formula intake.

Table 2. Effect Size Calculations for Intake (Provision minus Waste) of Beverages				
n per group	Minimum detectable difference in formula weight (g)	SD (g)	Power	Effect Size
30	6.80	9.71	0.80	0.70
40	5.83	9.71	0.80	0.60
45	5.44	9.71	0.80	0.56
50	5.15	9.71	0.80	0.53
53	4.95	9.71	0.80	0.51
55	4.86	9.71	0.80	0.50
60	4.66	9.71	0.80	0.48

With 53 subjects, we can detect a difference of 2.57 g (effect size or ES = 0.55, Table 3) between the RFPM's estimate and the weighed value for provision of powdered infant formula. This power analysis relied on variance estimates for condiments from our laboratory [10] which are similar to infant formula. This effect size of 2.57 grams reflects a very small amount of formula, indicating that the study is sufficiently powered. We recognize that predicting that no significant differences will be detected is problematic, though the proposed sample size and data analytic plan represents a viable alternative to equivalence tests that require very large sample sizes.

Table 3. Effect Size Calculations for Food Provision (Dry Weight)				
n per group	Minimum detectable difference in formula weight (g)	SD (g)	Power	Effect Size
30	3.51	4.68	0.80	0.75
40	3.00	4.68	0.80	0.64
45	2.81	4.68	0.80	0.60
50	2.62	4.68	0.80	0.56
53	2.57	4.68	0.80	0.55
55	2.53	4.68	0.80	0.54
60	2.39	4.68	0.80	0.51

9.2. Data Analysis

Paired dependent t-tests will determine if the RFPM has significant error; i.e., if RFPM estimates differ significantly from weighed food, and the Bland and Altman regression procedure will be conducted to determine if error variance differs over the amount of food provision, waste, and intake. Provision, waste, and intake will be compared using paired t-tests. Also, provision, waste, and intake will be compared using the Bland-Altman method. Statistical significance will be set at an alpha of 0.05.

10. SUBJECT PAYMENT

Subjects enrolled in this study will receive \$20. The full payment will be paid after completion of the 2 study visits. If participants drop out of the study or are withdrawn prior to completion of Visit 2, the participants will receive \$10 for the completion of Visit 1. The compensation is in line with all the other studies conducted at the PBRC.

11. REFERENCES

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APPENDIX B: INFORMED CONSENT

PBRC# 12035

Full approval by IRB received 9-20-12 

CONSENT TO PARTICIPATE IN A RESEARCH STUDY FOR AN ADULT INFORMED CONSENT - PART I

Remote Food Photography Method in Infants: A Pilot Study

Title of Study:

What you should know about a research study?

- We give you this consent form so that you may read about the purpose, risks and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary.
- By signing this consent form, you agree to participate in the study as it is described.

1- Who is doing the study?

Investigator Information:

Principal Investigators: Leanne M. Redman, Ph. D.
(225) 763-0947

Corby K. Martin, Ph. D.
(225) 763-2585

Medical Investigator: Frank Greenway, M.D.
Day Phone: (225) 763-2576
24-hr. Emergency Phone Nos.:
(225) 763-2672 (Weekdays 7:00 a.m.-4:30 p.m.)
(225) 765-4644 (After 4:30 p.m. and Weekends)

Co-Investigators:: Abby Duhé, B.S.
John Apolzan, Ph.D.
Shelly Ragusa, M.S.,LDN,RD

Dr. Redman and Dr. Martin direct this study, which is under the medical supervision of Dr. Greenway. We expect about 75 people from 1 site will be enrolled in this study. The study will take place over a period of 1 year. Your expected time in this study will be 2 weeks. There will be a maximum of 2 study visits at the Pennington Biomedical Research Center. This is a Pennington Biomedical Research Center study.



2- Where is the study being conducted?

This study takes place in the Outpatient Clinic and the Ingestive Behavior Laboratory of the Pennington Biomedical Research Center.

3- What is the purpose of this study?

The purpose of this study is to determine if digital photography on a Smartphone and the Remote Food Photography Method (RFPM) can estimate infant formula intake.

4- Who is eligible to participate in the study? Who is ineligible?

You are eligible to participate in this study if you:

- Are 18 years of age or older
- Are willing to complete 2 study visits at Pennington Biomedical Research Center

5- What will happen to you if you take part in the study?

If you agree to participate in the study, you will complete 2 study visits about 1 week apart. Study visits can be completed at any time of the day. An outline of the study tests performed at each visit is summarized in this table.

	Screening	Testing Visits	
	Telephone Screen	Visit 1	Visit 2
Eligibility Evaluation	X		
Informed Consent and HIPAA		X	
Height/Weight		X	
RFPM Training		X	
Infant Formula Preparation		X	X
Remote Food Photography Method		X	X
Directly Weighed Food Method		X	X

Visit 1- Approximately 1.5 hours, Non-Fasting

At this visit the following will occur:

- You will first sign the informed consent if you are willing to participate in the study.
- After you sign the consent, you will have your height and weight measured.
- You will be asked to complete a questionnaire about your demographics and caregiver status.
- You will be trained to take pictures using a Smartphone that we provide for you and send these pictures to us via a wireless network. This is called the Remote Food Photography Method (RFPM).
- After this training, you will prepare 8 bottles of infant formula with different amounts.
- To prepare each formula bottle, the following will occur:
 - You will use a formula scoop to scoop powdered formula.
 - After all powdered formula is in the formula bottle, you will take a picture of the formula bottle with powdered formula only.
 - We will weigh the formula bottle with the powdered formula in it.
 - You will pour water into the formula bottle and mix the formula.
 - You will take a picture of the formula bottle after the formula is prepared.
 - We will weigh the prepared formula bottle.
 - Then, we will remove some of the prepared formula from the bottle, and you will take a picture of the formula bottle after some of the formula is removed.
 - We will weigh the prepared formula bottle after you have taken a picture of the formula bottle after some of the formula is removed.
- You will schedule Visit 2 about 5 to 10 days after Visit 1.

**Visit 2- Approximately 30 minutes, Non-Fasting**

At this visit the following will occur:

- You will prepare 4 formula bottles at different sizes.
- To prepare each formula bottle, the following will occur:
 - You will use a formula scoop to scoop powdered formula.
 - After all powdered formula is in the formula bottle, you will take a picture of the formula bottle with powdered formula only.
 - We will weigh the formula bottle with the powdered formula in it.
 - You will pour water into the formula bottle and mix the formula.
 - You will take a picture of the formula bottle after the formula is prepared.
 - We will weigh the prepared formula bottle.
 - Then, we will remove some of the prepared formula from the bottle, and you will take a picture of the formula bottle after some of the formula is removed.
 - We will weigh the prepared formula bottle after you have taken a picture of the formula bottle after some of the formula is removed.

6- What are the possible risks and discomforts?

- **Body weight:** There is no risk to participants who record their body weight.
- **Height:** There is no risk to participants who record their height.
- **Self-report Questionnaires:** There are no anticipated risks from completing self-report questionnaires. It is estimated that the questionnaires will take up to 5 minutes to complete.
- **Formula Preparation:** There is no risk to participants who prepare or weigh infant formula.

7- What are the possible benefits?

We cannot promise any benefits from being in the study.

8- If you do not want to take part in the study, are there other choices?

You have the choice at any time not to participate in this research study. If you choose not to participate, any health benefits to which you are entitled will not be affected in any way.

9- If you have any questions or problems, whom can you call?

If you have any questions about your rights as a research volunteer, you should call the Institutional Review Board Office at 225/763-2693 or Dr. Steven Heymsfield, Executive Director of Pennington Biomedical Research Center at 225/763-2513. If you have any questions about the research study, contact Leanne Redman (PI) at 225/763-0947 or Corby Martin (PI) at 225/763-2585. If you think you have a research-related injury or medical illness, you should call Dr. Greenway at 225/763-2576 during regular working hours. After working hours and on weekends you should call the answering service at 225/765-4644. The on-call physician will respond to your call.

10- What information will be kept private?

Every effort will be made to maintain the confidentiality of your study records. However, someone from the Pennington Biomedical Research Center may inspect and/or copy the medical records related to the study. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

11- Can your taking part in the study end early?

Dr. Redman, Dr. Martin, or Dr. Greenway can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include inability to comply with study demand. The sponsor of the study may end the study early.

12- What if information becomes available that might affect your decision to stay in the study?

During the course of this study there may be new findings from this or other research which may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13- What charges will you have to pay?

None

14- What payment will you receive?

If you agree to take part, we will pay you \$20. If you drop out of the study or are withdrawn prior to completion of Visit 2, you will receive \$10 as compensation for completing Visit 1. Your check will be requested from the LSU payroll department when you complete the study or at the appropriate milestone if you are compensated during the course of the study. It usually takes about 2-3 weeks for it to arrive at Pennington Biomedical Research Center.

15- Will you be compensated for a study-related injury or medical illness?

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should you require ongoing medical treatments, they must be provided by community physicians and hospitals.

16- HIPAA

Records that you give us permission to keep, and that identify you, will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in records disclosed outside of Pennington Biomedical Research Center. For records disclosed outside of Pennington Biomedical Research Center, you will be assigned a unique code number.

**17- Signatures**

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a copy of the signed consent form.

With my signature, I also acknowledge that I have been given either today or in the past a copy of the Notice of Privacy Practices for Protected Health Information.

Printed Name of Volunteer

Signature of Volunteer

Date

Date of Birth of Volunteer

Signature of Person Administering Informed Consent

Date

Leanne M. Redman
Principal Investigator

Corby K. Martin
Principal Investigator

Frank Greenway
Medical Investigator

APPENDIX C: HIPAA AUTHORIZATION

PENNINGTON BIOMEDICAL RESEARCH CENTER (PBRC) INSTITUTIONAL REVIEW BOARD

AUTHORIZATION FOR USE AND DISCLOSURE OF PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES INFORMED CONSENT – PART II

(Instructions for Investigators: This form must be reviewed and signed by subjects participating in research/clinical trials that require a signed Informed Consent. These documents should be kept together. A copy of this Authorization and the Informed Consent must be given to the subject and/or his/her representative.)

Title of Research Project: **Remote Food Photography Method in Infants: A Pilot Study**

Principal Investigator: **Leanne Redman, Ph.D. and Corby Martin, Ph.D.**

IRB Number: **12035 BABY BOTTLE**

I hereby request and authorize the PBRC to use and disclose protected health information from the record(s) of:

Subject's Name/Address: _____

Birth Date: ____/____/____

Social Security Number: _____

Specifically, I request and authorize any part of my health information relevant to the research project, identified above and in the Informed Consent document, to be used and/or disclosed to the Principal Investigator identified above or his/her designee, in connection with the research project. I understand that this may include information relating to: Human Immunodeficiency Virus ("HIV") infection or Acquired Immunodeficiency Syndrome ("AIDS"); treatment for or history of drug or alcohol abuse; and/or mental or behavioral health or psychiatric care.

I understand that copies of the records indicated above will be:

- Used by employees of PBRC including researchers and treatment providers, and/or other members of its workforce.
- Disclosed to government officials or government agencies, study sponsors, study monitors, or others responsible for oversight of the research project.
- Sent to collaborating researchers outside PBRC if and to the extent indicated in the attached Informed Consent document(s).

I understand that by signing this form, I will allow PBRC and its researchers to use or disclose my health information in connection with the attached Informed Consent and for the purpose of the research that is described in the Informed Consent. For example, the researchers may need the information to verify that I am eligible to participate in the study, or to monitor the results, including expected or unexpected side effects or outcomes. Other University and government officials, safety monitors, and study sponsors may need the information to ensure that the study is conducted properly. I understand that any privacy rights not specifically mentioned in this Authorization are

contained in the Notice of Privacy Practices that I received or will receive from the Principal Investigator or at the facility that I attend.

I understand that I may revoke this authorization at any time, except to the extent that PBRC has already relied on the authorization, by sending or transmitting of a facsimile, a written notice to the contact person listed in the attached Informed Consent document(s).

I understand that if my information already has been included in a research database or registry as described in the attached Informed Consent document(s), PBRC considers itself to have relied on it, and therefore my information will not be removed from those repositories, unless I request for it to be removed. Unless otherwise revoked, I understand that this authorization will not expire during the length of the research study. I understand that if I do not sign this form, I will not be able to participate in the above research study or receive the study-related interventions, but that PBRC cannot otherwise condition treatment on my signing this form.

While the research study is in progress, my right to access any research records or results that are maintained by the facility may be suspended until the research study is over. If my access is denied, I understand that it will be reinstated at the end of the research study.

I understand the information disclosed by this authorization may be subject to re-disclosure by the recipient and no longer be protected by the Health Insurance Portability and Accountability Act. The PBRC facility, its employees, officers, and physicians are hereby released from any legal responsibility or liability for disclosure of the above information to the extent indicated and authorized herein.

I UNDERSTAND THAT THIS AUTHORIZATION SUPERSEDES ANY CONTRARY INFORMATION IN ANY OTHER DOCUMENTS I HAVE SIGNED RELATED TO THE ATTACHED STUDY.

Signature of Subject or Subject's Legal Representative

Date

Printed Name of Legal Representative (if any): _____

Representative's Authority to Act for Subject (e.g., relationship to subject): _____

Verification of Representative's Authority: () viewed driver's license () viewed Power of Attorney

() viewed other _____ (specify)

APPENDIX D: BABY BOTTLE QUESTIONNAIRE



PBRC Participant label

Baby Bottle Questionnaire

Please complete the following questions by filling in the bubble that corresponds to the answer that most closely applies to you.

Demographic Information

1. Education (Fill in the box that corresponds to the highest level completed)
☐ Grades 0-8 ☐ Some High School ☐ High School Diploma/GED ☐ 1-3 years college ☐ College Degree ☐ Post graduate degree
2. What is your approximate household income?
☐ Less than 10,000/year ☐ 10,000-29,999/year ☐ 30,000-49,999/year ☐ 50,000-79,999/year ☐ 80,000-99,999/year ☐ 100,000-129,999/year ☐ 130,000 and above
3. Please indicate your present employment status.
☐ Full time (at least 35 hrs/ week) ☐ Part time (at least 20 hrs/week) ☐ Retired ☐ Unemployed or Part (<20 hrs/week) ☐ Medical disability

Caregiver Information

4. Are you a parent or guardian?
☐ Yes ☐ No
➤ If you answered yes, how many children are you guardian of?
☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5
➤ What are their respective age ranges? (Mark all that apply)
☐ 0-1 ☐ 2-4 ☐ 5-10 ☐ 11-18 ☐ 19-30 ☐ 31-50 ☐ 51 or older
5. Have you cared for an infant within the last 12 months? (Includes babysitting)
☐ Yes ☐ No
If you answered yes, what is your relationship to the infant(s) you have cared for? (Mark all that apply)
☐ Parent ☐ Grandparent ☐ Sibling ☐ Aunt/Uncle ☐ Cousin ☐ Nanny/babysitter ☐ Other
6. Have you prepared infant formula bottles using powdered formula within the last 12 months?
☐ Yes ☐ No

APPENDIX E: VISIT 1 DIRECTLY WEIGHED FOODS DATASHEET

Pennington Biomedical Research Center
0480-0000 : Baby Bottle
Directly-Weighed Foods Form (Clinic Visits)



**Place Identification
Label Here**

Please circle AM or PM when recording start time

Visit: **V1**

Randomization Group #

|_|_|_|

Randomization Group #

|_|_|_|

Trial 1

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 5

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 2

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 6

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 3

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 7

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 4

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

Trial 8

Start Time

|_|_|_|:|_|_|_| AM PM

Bottle with Powdered Formula Weight

|_|_|_|.|_|_|(g)

Prepared Formula Bottle Weight

|_|_|_|.|_|_|(g)

Waste Randomization

|_|_|_|%

Waste Bottle Weight

|_|_|_|.|_|_|(g)

APPENDIX F: VISIT 2 DIRECTLY WEIGHED FOODS DATASHEET

Pennington Biomedical Research Center
0480-0000 : Baby Bottle
Directly-Weighed Foods Form (Clinic Visits)



**Place Identification
Label Here**

Please circle AM or PM when recording start time

Visit: V2

Randomization Group # |__|__|__|

Trial 1

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 2

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 3

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 4

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Randomization Group # |__|__|__|

Trial 5

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 6

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 7

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

Trial 8

Start Time |__|__|:|__|__| AM PM

Bottle with Powdered Formula Weight |__|__|__|. |__| (g)

Prepared Formula Bottle Weight |__|__|__|. |__| (g)

Waste Randomization |__|__|__| %

Waste Bottle Weight |__|__|__|. |__| (g)

APPENDIX G: PBRC CONCURRENT MEDICATIONS DATASHEET

Pennington Biomedical Research Center
Concurrent Medications/Non-Drug Therapies
CMeds (Clinic Visits)

Label

C-med #	Date Reported DD/MM/YYYY	Visit	Medication Or Therapy	Single Dose Ex. (500)	Unit Ex. (Mg, mcg)	Frequency	Route	Medical Indication Reason	Start Date DD/MM/YYYY	Stop Date DD/MM/YYYY	Ongoing at end of study? Y/N	Given for AE *Complete AE Log Y/N

APPENDIX H: PBRC ANTHROPOMETRIC DATASHEET

Pennington Biomedical Research Center



Place Identification
Label Here

Screening #1 Part A			Anthropometric Data	
Height & Weight				
Measured Height (135–200cm)			Measured Weight (41–150kg)	
1) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm			1) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> kg	
2) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm			2) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> kg	
Avg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm			Avg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> kg	
Operator ID	Avg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> in		Avg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> lbs	
Body Mass Index				
Body Mass Index = $\frac{\text{Body Weight in kg}}{\text{Height in m}^2}$			BMI = <input type="text"/> <input type="text"/> . <input type="text"/>	
Circumferences				
Upper Arm Circum (22–50cm) <input type="text"/> <input type="text"/> . <input type="text"/> cm		Waist Circum (50–140cm) 1) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm		
Hip Circum. <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm		Waist Circum (50–140cm) 2) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/> cm		
				Operator ID
Blood Pressure				
<i>Cuff size used</i>	<i>Cuff</i>	<i>Cuff size</i>	<i>Measured Blood Pressure</i>	
			SBP (90–180)	DBP-V (60–100)
<input type="checkbox"/>	Small Adult/Child	< 24 cm	1) <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> mm. Hg	
<input type="checkbox"/>	Regular Adult	> 24 - 32 cm	2) <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> mm. Hg	
<input type="checkbox"/>	Large Adult	> 32 - 41 cm	3) <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> mm. Hg	
<input type="checkbox"/>	Thigh	> 41.0 cm	Avg) <input type="text"/> <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> <input type="text"/> mm. Hg	
<input type="checkbox"/>	Large Long	> 41.0 cm		
Resting 60-second pulse (50–100)		<input type="text"/> <input type="text"/> <input type="text"/> / 60 sec		
				Operator ID
Other				
Finger Stick Glucose (40–500) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>				
				Operator ID

THE VITA

Abby Francis Duhé, a native of Baton Rouge, Louisiana, attended high school at St. Joseph's Academy, and graduated with honors in May 2007. Abby began her undergraduate work in biological engineering at Louisiana State University in August 2007. She earned a Bachelor's of Science degree in biological engineering in December 2011. Upon completion of her undergraduate work, Abby also earned a minor in nutritional sciences. Following graduation, Abby began graduate school in the School of Human Ecology with a concentration in human nutrition at Louisiana State University. While attending graduate school, she worked as a graduate research assistant under Dr. Leanne Redman in the Reproductive Endocrinology and Women's Health laboratory at Pennington Biomedical Research Center. Abby plans to earn a Master's of Science in the School of Human Ecology with a concentration in human nutrition in December 2013. Upon completing her master's program, Abby hopes to continue her employment in the Reproductive Endocrinology and Women's Health laboratory at Pennington Biomedical Research Center.